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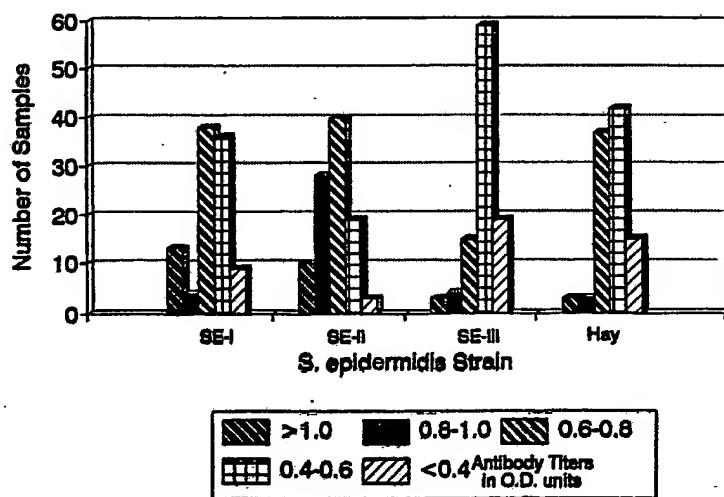
(57) Abstract

The invention relates to the identification, making, and isolation of immunoglobulin and antigen that is useful to prevent, diagnose, or threat Staphylococcus infections. The invention further relates to an *in vivo* animal model for testing the efficacy of pharmaceutical compositions, including the pharmaceutical compositions of immunoglobulin and isolated antigen described herein.

## DESCRIPTION
### Broadly Reactive Opsonic Antibodies That React With Common Staphylococcal Antigens

### GOVERNMENT INTEREST
The invention described herein may be manufactured, licensed and used by or for governmental purposes without the payment of any royalties to us thereon.

### FIELD OF THE INVENTION
This invention relates to immunoglobulin (including polyclonal antibodies and monoclonal antibodies) and isolated antigen used to prevent, diagnose, or treat Staphylococcus infections. This invention also relates to an animal model used to determine the efficacy of pharmacological compositions against infectious agents including, but not limited to, Staphylococcus infections.

### BACKGROUND OF THE INVENTION
Over the last two decades, Staphylococcus infections have become important causes of human morbidity and mortality, particularly in hospitalized patients. Because of their prevalence on the skin and mucosal linings, Staphylococci are ideally situated to produce infections, both localized and systemic. Debilitated or immunosuppressed patients are at extreme risk of systemic infection.

The Staphylococcus species most frequently pathogenic in humans are Staphylococcus aureus and Staphylococcus epidermidis, and each includes a number of serotypes. Both groups have developed resistance to antibiotics, the current treatment of choice. In recent years, S. epidermidis has become a major cause of nosocomial infection in patients whose treatments include the placement of foreign objects such as cerebrospinal fluid shunts, cardiac valves, vascular catheters, joint prostheses, and other implants into the

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body. S. epidermidis is also a common cause of post-
operative wound infections and peritonitis in patients with
continuous ambulatory peritoneal dialysis. One form of
treatment for kidney failure entails the introduction of
large volumes of peritoneal dialysis fluid into the perito-
neal cavity which carries the risk of frequent and recurrent
infections. In a similar manner, patients with impaired im-
munity and those receiving parenteral nutrition through cen-
tral venous catheters are at high risk for developing S.
epidermidis sepsis as well (C.C. Patrick, J. Pediatr.,
116:497 (1990)).

　　　　S. epidermidis has also become a common cause of neona-
tal nosocomial sepsis. Infections frequently occur in prema-
ture infants that have received parenteral nutrition which
can be a direct or indirect source of contamination. Such
infections are difficult to treat for a variety of reasons.
Resistance to antibiotics is common. In one study, the ma-
jority of staphylococci isolated from blood cultures of sep-
tic infants were multiply resistant to antibiotics (A. Fleer
et al., Pediatr. Infect. Dis. 2:426 (1983)). Stimulation of
the immune system provides little relief because such infants
have impaired immunity resulting from deficiencies in anti-
bodies, complement, and neutrophil function. Moreover, lipid
infusion, which is now a standard ingredient of parenteral
nutrition therapy, further impairs the already poor immune
response of these infants to bacterial infection (G.W.
Fischer et al., Lancet 2:819 (1980)).

　　　　Supplemental immunoglobulin therapy has been shown to
provide some measure of protection against certain encapsu-
lated bacteria such as Hemophilus influenzae and Streptococ-
cus pneumoniae. Infants who are deficient in antibody are
susceptible to infections from these bacteria and bacteremia
and sepsis are common. When anti-Streptococcal and anti-
Hemophilus antibodies are present, they provide protection by
promoting clearance of the respective bacteria from the

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blood. In the case of antibody to Staphylococcus, the potential use of supplemental immunoglobulin to prevent or treat infection has been much less clear.

Early studies of Staphylococcus infections focused on the potential use of supplemental immunoglobulin to boost peritoneal defenses, such as opsonic activity, in patients receiving continuous ambulatory peritoneal dialysis. Standard intravenous immunoglobulin (IVIG) was shown to have lot to lot variability for opsonic activity to S. epidermidis (L.A. Clark and C.S.F. Easmon, J. Clin. Pathol. 39:856 (1986)). In this study, one third of the IVIG lots tested had poor opsonization with complement, and only two of fourteen were opsonic without complement. Thus, despite the fact that the IVIG lots were made from large plasma donor pools, good opsonic antibody to S. epidermidis was not uniformly present. Moreover, this study did not examine whether IVIG could be used to prevent or treat S. epidermidis infections or bacterial sepsis.

Recent studies have associated coagulase-negative Staphylococcus bacteremia, such as S. epidermidis, as the most common species causing bacteremia in neonates receiving lipid emulsion infusion (J. Freeman et al., N. Engl. J. Med. 323:301 (1990)). These neonates had low levels of opsonic antibody to S. epidermidis despite the fact that the sera had clearly detectable levels of IgG antibodies to S. epidermidis peptidoglycan (A. Fleer et al., J. Infect. Dis. 2:426 (1985)). This was surprising because anti-peptidoglycan antibodies were presumed to be the principal opsonic antibodies. Thus, while suggesting that neonatal susceptibility to S. epidermidis might be related to impaired opsonic activity, these studies also suggested that many antibodies directed against S. epidermidis are not opsonic and would not be capable of providing protection when given passively to neonates.

Recently, an antigen binding assay was used to analyze IgG antibody to S. epidermidis in patients with uncomplicated bacteremia and those with bacteremia and endocarditis (F.

Esp rsen et al., Arch. Intern. Med. 147:689 (1987)). This
assay used an ultrasonic extract of S. epidermidis to id n-
tify S. epidermidis specific IgG. None of the patients with
uncomplicated bacteremia had IgG antibodies to S.
epidermidis. These data suggest that IgG does not provide
effective eradication of S. epidermidis from the blood. In
addition, 89% of bacteremic patients with endocarditis devel-
oped high levels of IgG to S. epidermidis. In these pa-
tients, IgG was not protective since high levels of IgG anti-
body were associated with serious bacteremia and endocardi-
tis. Based on these studies, the protective role of IgG in
S. epidermidis sepsis and endocarditis was questionable, es-
pecially in the presence of immaturity, debilitation,
intralipid infusion, or immunosuppression.

Animal studies in the literature that demonstrated im-
munoglobulin protection against Staphylococcus infections
have shown strain specificity by enzyme-linked immunosorbent
assays (ELISA) and have utilized normal adult mice in protec-
tion studies. These studies do not mimic the disease as ob-
served in humans. Animal models typically have used mature
animals with normal immunity and then given unusually viru-
lent strains or overwhelming-challenge doses of bacteria.
Human patients are generally immunologically immature or de-
bilitated. Human patients also get somewhat indolent infec-
tions with low virulence pathogens such as S. epidermidis
with death usually attributable to secondary complications.
Models that have used unusual strains or overwhelming bacte-
rial doses, generally induce rapid fulminant death. These
are important factors since antibodies generally work in con-
cert with the host cellular immune system (neutrophils, mono-
cytes, macrophages and fixed reticuloendothelial system).
The effectiveness of antibody therapy may therefore be depen-
dent on the functional immunologic capabilities of the host.
To be predictive, animal models must closely emulate the
clinical condition in which the infection would occur and
capture the setting for th rapy. Moreov r, the animal stud-
ies have yield d inconsistent results.

One model has been reported which used an unusually virulent strain of S. epidermidis. Infected-mature mice d - veloped 90 to 100% mortality within 24 to 48 hours (K. Yoshida et al., Japan. J. Microbiol. 20:209 (1976)). Antibody to S. epidermidis surface polysaccharide was protective in these mice. Protection was shown to occur with an IgM fraction, but not the IgG fraction (K. Yoshida and Y. Ichiman, J. Med. Microbiol. 11:371 (1977)). This model, however, presents a pathology which is very different from that seen in typically infected patients. Intraperitoneally-challenged mice developed symptoms of sepsis within minutes of receiving the injection and died in 24 to 48 hours. This particular pathology is not observed in Staphylococcus infected humans. The highly virulent strain of S. epidermidis may represent an atypical type of infection. Moreover, isolates of S. epidermidis from infected humans did not kill mice in this model.

In 1987, these animal studies were extended to include the evaluation of antibodies in human serum against selected virulent strains of S. epidermidis (Y. Ichiman et al., J. Appl. Bacteriol. 63:165 (1987)). In contrast to the previous data, protective antibody was found in the IgA, IgM and IgG immunoglobulin fractions. A definitive role for any single class of immunoglobulin (IgG, IgM, IgA) could not be established.

In this animal model, normal adult mice were used and mortality was determined. Death was considered to be related to the effect of specific bacterial toxins, not sepsis (K. Yoshida et al., Japan J. Microbiol. 20:209 (1976)). Most clinical isolates did not cause lethal infections, and quantitative blood cultures were not done. Moreover, this study provided little insight as to whether antibody could successfully prevent or treat S. epidermidis sepsis in immature or immunosuppressed patients.

In a later study, serotype specific antibodies directed against S. epidermidis capsular polysaccharides were tested in the animal model. Results showed that serotype-specific

antibodies were protective, but that each antibody was directed against one serotype as measured by ELISA. Prot ction was equally serotype specific. Protection against heterologous strains did not occur. In addition, it was concluded that protection was afforded by the IgM antibody.

In short, there has been no compelling evidence that IVIG would be effective to treat S. epidermidis infections or sepsis, particularly where the patients are immature or immune suppressed or where multiple S. epidermidis serotypes are involved. Thus, for example, a recent and extensive review of the pathogenesis, diagnosis, and treatment of S. epidermidis infections does not include immunoglobulin as a potential prophylactic or therapeutic agent (C.C. Patrick, J. Pediatr. 116:497 (1990)).

In addition, no animal model has been developed which is comparable to human patients with S. epidermidis infections, particularly those who are immature or immune suppressed. This is critical because these patients have low levels of complement as well as impaired neutrophil and macrophage function. Thus, even if opsonic activity of immunoglobulin may appear adequate under optimal conditions in vitro, protection may not occur in patients such as newborn babies or cancer patients. Moreover, previous models have been shown to be unsatisfactory in that they used animals which did not possess similar risk factors to the typical high-risk human patient.

At present, antibiotic therapy is the treatment of choice for the prevention and cure of Staphylococcus infections in humans. Although new antibiotics are constantly being developed, it has become increasing clear that antibiotic therapy alone is insufficient. The data regarding passive vaccinations with immunoglobulin is at best unclear. The animal models on which this therapy has been attempted bear little relationship to human infections and as yet, have produced no definitive solutions.

## SUMMARY OF THE INVENTION

The present invention overcomes th problems and disadvantages associated with current strategies and designs and provides a new therapy for the treatment and prevention of Staphylococcus infections. As broadly described herein, this invention relates to the discovery that there are broadly reactive opsonic antibodies that react with common staphylococcal antigens from which vaccines, pharmaceutical compositions, and diagnostic aids can be created for the treatment and prevention of Staphylococcus infections in both man and animals. The invention includes immunoglobulin, which may be found in individual samples or pools of serum, plasma, whole blood, or tissue, isolated immunoglobulin, which may be polyclonal antibodies or monoclonal antibodies, methods for making polyclonal and monoclonal antibodies, isolated antigen, methods for making isolated antigen, pharmaceutical compositions comprising isolated immunoglobulin or isolated antigen, and methods for the prophylactic or therapeutic treatment of a patient with the pharmaceutical compositions. In addition, this invention also comprises an animal model to evaluate the efficacy of pharmaceutical compositions *in vivo*, diagnostic aids and methods for the detection of a Staphylococcus infection, and methods to detect pharmaceutical compositions in biological samples, including pharmaceutical compositions as described herein.

In accord with this invention, and as broadly described herein, a first object of the present invention is the identification of immunoglobulin, which may be from individual samples or pools of serum, plasma, whole blood, or tissue for the treatment of a Staphylococcus infection. Immunoglobulin is identified by performing a first assay to identify immunoglobulin which is reactive with a preparation of a first Staphylococcus organism, performing a second assay to identify immunoglobulin which is reactive with a preparation of a second Staphylococcus organism, and selecting immunoglobulin which is reactiv with th preparations from both the first

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and second Staphylococcus organisms. Reactivity is deter-
mined in immunological assays which may be binding assays,
opsonization assays, or clearance assays. Preferably, the
preparations of the first and the second Staphylococcus or-
ganisms are derived from different serotypes or different
species, such as S. epidermidis and S. aureus, and more pref-
erably, the first preparation is from S. epidermidis (Hay,
ATCC 55133).

In accord with this invention, and as broadly described
herein, a second object of the present invention is the iso-
lation of immunoglobulin which reacts in a first assay with a
preparation of a first Staphylococcus organism and in a sec-
ond assay with a preparation of a second Staphylococcus or-
ganism. The invention includes the isolation of polyclonal
antibodies, which are produced by introducing a preparation
of a Staphylococcus organism into an animal and isolating
serum, and monoclonal antibodies, which are produced by hy-
bridoma technology. Preferably, the isolated immunoglobulin
is of the IgG fraction or isotype, but isolated im-
munoglobulin is not restricted to any particular fraction or
isotype and may be IgG, IgM, IgA, IgD, IgE, or any combina-
tion thereof. It is also preferable that the isolated im-
munoglobulin be purely or antigenically human immunoglobulin,
which may be made directly by the fusion of human antibody
producing cells with human antibody producing cells or by the
substitution of human DNA sequences for some of the nonhuman
DNA sequences which code for the antibody while retaining the
antigen binding ability of the original antibody molecule.
Isolated immunoglobulin may be used to treat patients in-
fected with or suspected of being infected with a Staphylo-
coccus organism, and prophylactically to prevent possible
Staphylococcus infections. Further, isolated immunoglobulin
may be used prophylactically to treat objects, articles, in-
struments and appliances which are introduced into a patient
and are suspected of becoming infected with and introducing a
Staphylococcus infection into a patient.

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In accord with this invention, and as broadly described
herein, a third object of the pr s nt invention is isolated
antigen which generates an antibody that reacts in a first
assay with a preparation of a first Staphylococcus organism
and in a second assay with a preparation of a second Staphy-
lococcus organism.  As used herein, isolated antigen means
any single antigen, any mixture of different antigens, or any
combination of antigens which are separated from one or more
different Staphylococcus organisms.  Isolated antigen may be
used directly as a pharmaceutical composition, such as a Sta-
phylococcus vaccine, and indirectly to generate antibodies,
both monoclonal and polyclonal, to treat or prevent Staphylo-
coccus infections in man and animals.

In accord with this invention, and as broadly described
herein, a fourth object of the present invention is the iden-
tification of an animal model to evaluate the efficacy of
pharmaceutical compositions in vivo.  This animal model may
be broadly applied to test the efficacy of a wide variety of
pharmaceuticals against infection by bacteria, preferably
Staphylococci, but also viruses, parasites and fungi.  It
comprises the administration of a pharmaceutical composition,
an immune suppressant, and an infectious agent to an immature
animal, and evaluating whether the pharmaceutical composition
reduces mortality of the animal or enhances clearance of the
infectious agent from the animal.  The pharmaceutical compo-
sition may be isolated immunoglobulin or isolated antigen of
the invention as described herein, and may be administered
prophylactically or therapeutically.

In accord with this invention, and as broadly described
herein, a fifth object of the present invention comprises
diagnostic aids and methods for the diagnosis of Staphylococ-
cus infections which employ as reagents isolated im-
munoglobulin, isolated antigen or preparations of Staphylo-
coccus organisms.

These diagnostic aids can be used to identify which
laboratory isolat s of Staphylococci isolated from a patient

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or other samples are pathogenic.  In addition, these diagnostic aids could be used to identify in body fluids antigens associated with pathogenic Staphylococci.  These reagents are also of use to detect pharmaceutical compositions in biological samples to analyze the utility of a particular pharmaceutical composition, including pharmaceutical compositions described herein.  In addition, these reagents are also highly useful as tools to examine the biology of the Staphylococcus organism and its course of infection experimentally in a laboratory setting.

Other objects and advantages of the present invention will be set forth in part in the description which follows, and in part will be obvious from this description, or may be learned from the practice of this invention.  The accompanying drawings and tables, which are incorporated in and constitute a part of this specification, illustrate and, together with this description, serve to explain the principle of the invention.

BRIEF DESCRIPTIONS OF THE DRAWINGS

Figure 1.    Antibody titers of human plasma tested for binding to S. epidermidis serotypes I, II, III, and Hay.

Figure 2.    Pre- and post-immunization ELISA titers of sera from rabbits immunized with a TCA prepared preparation of S. epidermidis (Hay, ATCC 55133) tested for binding to S. epidermidis serotypes I, II, III and Hay.

Figure 3.    Pre- and post-immunization ELISA titers of sera from rabbits immunized with a whole cell preparation of S. epidermidis (Hay, ATCC 55133) tested for binding to S. epidermidis serotypes I, II, III and Hay.

Figure 4.    Neutrophil mediated opsonization assay of S. epidermidis, S. aureus, and Streptococcus agalactiae organisms using immunoglobulin which has be n selected for the ability to bind to a

preparation of <u>S</u>. <u>epidermidis</u>, and selected im-
munoglobulin which has been pr absorbed with a
preparation of <u>S</u>. <u>epidermidis</u>. Negative control
is neutrophils plus complement alone.

Figure 5.    Opsonic activity measured as percent bactericidal
response of rabbit serum pre- and post-
immunization with a TCA prepared preparation of
<u>S</u>. <u>epidermidis</u> (Hay, ATCC 55133) against <u>S</u>.
<u>epidermidis</u> serotypes I, II, III, and Hay.

Figure 6.    Opsonic activity measured as percent bactericidal
response of rabbit serum pre- and post-
immunization with a whole cell preparation of <u>S</u>.
<u>epidermidis</u> (Hay, ATCC 55133) against <u>S</u>.
<u>epidermidis</u> serotypes I, II, III, and Hay.

Figure 7.    Opsonic activity of pre- and post-immunization
serum with TCA prepared or whole cell preparation
of <u>S</u>. <u>epidermidis</u> (Hay, ATCC 55133) against <u>S</u>.
<u>aureus</u> type 5. Opsonic assays were calculated
using two dilutions of the reaction mixture prior
to subculturing onto solid agar.

Figure 8.    Bacteremia levels of <u>S</u>. <u>epidermidis</u> in samples of
blood from suckling rats treated with either
high-titer immunoglobulin, selected for the abil-
ity to bind to a preparation of <u>S</u>. <u>epidermidis</u>,
or unselected low-titer immunoglobulin.

Figure 9.    Effect of directed (selected high-titer) im-
munoglobulin and saline injections on survival in
suckling rats treated with intralipid plus <u>S</u>.
<u>epidermidis</u>.

Figure 10.   Effect of directed (selected high-titer) im-
munoglobulin, directed immunoglobulin preabsorbed
with a preparation of <u>S</u>. <u>epidermidis</u>, and saline
injections on survival in suckling rats treated
with intralipid plus <u>S</u>. <u>epidermidis</u>.

Figure 11.   Effect of directed (selected high-titer) im-
munoglobulin, directed immunoglobulin preabsorbed
with a pr paration of <u>S</u>. <u>epid rmidis</u>, and saline

injections on bacteremia levels in the blood of
suckling rats treated with intralipid plus S.
epidermidis.

Figure 12.    Relationship between opsonic activity measured in
vitro and survival in the suckling rat model with
directed (selected high-titer) immunoglobulin,
unselected low-titer immunoglobulin, directed
immunoglobulin which has been preabsorbed with a
preparation of S. epidermidis, and saline.


## DESCRIPTION OF PREFERRED EMBODIMENTS

To achieve the objects and in accordance with the pur-
pose of the invention, as embodied and broadly described
herein, the present invention comprises the identification,
making, and isolation of immunoglobulin and antigen useful to
prevent, diagnose, or treat Staphylococcus infections. The
invention further comprises an in vivo animal model for test-
ing the efficacy of pharmaceutical compositions, including
compositions of immunoglobulin and preparations described
herein.

One embodiment of the present invention is a method of
identifying immunoglobulin for the treatment of a Staphylo-
coccus infection, comprising the steps of performing a first
assay to identify immunoglobulin which is reactive with a
preparation of a first Staphylococcus organism, performing a
second assay to identify immunoglobulin which is reactive
with a preparation of a second Staphylococcus organism, and
selecting immunoglobulin which is reactive with the prepara-
tions from both the first and second Staphylococcus organ-
isms. The immunoglobulin may be derived from pooled or indi-
vidual samples of plasma, serum, whole blood, or tissue such
as placenta. Although the isolation of immunoglobulin is not
required, if it is determined to be necessary, such proce-
dures are well-known to those of ordinary skill in the art.
The first and second assays may be any immunological assays
and preferably are binding assays, opsonization assays,

clearance assays, or any combination of these assays.  Pref-
erably, the first and second Staphylococcus organisms are of
different serotypes or of different species.

The first and second preparations of a Staphylococcus
organism may be any preparations of a Staphylococcus organism
including intact cells, cells fractionated by chemical or
physical means, or cell extracts and is preferably a whole-
cell or cell surface extract.  It is preferred that one
preparation is from S. epidermidis (Hay, ATCC 55133).  A
preparation of a Staphylococcus organism is comprised of
polysaccharides, proteins, lipids and other bacterial cell
components.  It is preferred that the preparation is a
polysaccharide and protein preparation, i.e., a preparation
that predominantly contains mixtures or combinations of
polysaccharides, proteins and glycoproteins.     A suitable
preparation may be prepared by isolating a culture of bacte-
rial cells of Staphylococcus epidermidis (Hay, ATCC 55133),
suspending the isolated cells in a mixture comprised of a
solution of trichloroacetic acid, stirring the mixture at
approximately 4°C, centrifuging the mixture and saving the
resulting supernatant, combining the supernatant with an al-
cohol, preferably absolute ethanol, incubating the alcohol-
supernatant combination at approximately 4°C to precipitate a
preparation, and isolating the precipitated preparation.

One preferred assay is a binding assay wherein im-
munoglobulin is reacted with a preparation of a Staphylococ-
cus organism.  The binding assay is preferably an enzyme-
linked immunosorbent assay (ELISA), or a radio immune assay
(RIA), but may also be an agglutination assay, a
coagglutination assay, a colorimetric assay, a fluorescent
binding assay, or any other suitable binding assay.  It may
be performed by competitive or noncompetitive procedures with
results determined directly or indirectly.

In the binding assay, the preparation of a Staphylococ-
cus organism may be fixed to a solid support which may be any
surface suitable for supporting the preparation.  Preferably,
the solid support is a glass or plastic plate, well, bead,

micro-bead, paddle, propeller, or stick, and is most prefer-
ably a titration plate. The fixed preparation is incubated
with immunoglobulin, which may be isolated or within a bio-
logical fluid, and the amount of binding determined. A posi-
tive reaction occurs when the amount of binding observed is
greater than the amount of binding of a negative control. A
negative control is any sample which is known not to contain
antigen specific immunoglobulin. Positive binding may be
determined from a simple positive/negative reaction or from
the calculation of a series of reactions. This series may
include samples which contain measured amounts of im-
munoglobulin that specifically bind to the fixed antigen cre-
ating a standard curve from which the amount of antigen spe-
cific immunoglobulin in an unknown sample can be determined.
Alternatively, the assay may be performed in substantially
the same way with antibody fixed to the solid support and
immunoglobulin identified by its ability to be retained to a
preparation bound to the fixed antibodies.

Another preferred assay is an opsonization assay which
may be a colorimetric assay, a chemilumenescent assay, a
fluorescent or radiolabel uptake assay, a cell mediated bac-
tericidal assay, or any other appropriate assay which mea-
sures the opsonic potential of a substance. In an
opsonization assay, an infectious agent, a eukaryotic cell,
and the to be tested opsonizing substance or an opsonizing
substance plus a purported opsonizing enhancing substance,
are incubated together. Most preferably, the opsonization
assay is a cell mediated bactericidal assay. In this in
vitro assay an infectious agent, typically a bacterium, a
phagocytic cell and an opsonizing substance, in this case
immunoglobulin, are incubated together. Although any eukary-
otic cell with phagocytic or binding ability may be used in a
cell mediated bactericidal assay, a macrophage, a monocyte, a
neutrophil or any combination of these cells is preferred.
Complement proteins may be included to observe opsonization
by both the classical and alternate pathways.

The opsonic ability of immunoglobulin is determined
from the amount or number of infectious ag nts that remain
after incubation. In a cell mediated bactericidal assay,
this is accomplished by comparing the number of surviving
bacteria between two similar assays, only one of which con-
tains the purported opsonizing immunoglobulin or by measuring
the numbers of viable organisms before and after incubation.
A reduced number of bacteria after incubation in the presence
of immunoglobulin indicates a positive opsonizing ability.
In the cell mediated bactericidal assay, positive
opsonization is determined by culturing the incubation mix-
ture under appropriate bacterial growth conditions. Any sig-
nificant reduction in the number of viable bacteria comparing
pre- and post-incubation samples or between samples which
contain immunoglobulin and those that do not is a positive
reaction.

Another preferred method of identifying immunoglobulin
for the treatment of a Staphylococcus infection employs a
clearance assay. Preferably, the clearance assay is con-
ducted in an animal model. A particularly useful animal
model comprises the steps of administering a pharmaceutical
composition, an immune suppressant, and a Staphylococcus or-
ganism to an immature animal, and evaluating whether the
pharmaceutical composition reduces mortality of the animal or
enhances clearance of the Staphylococcus organism from the
animal. This assay may use any immature animal including the
rabbit, the guinea pig, the mouse, the rat, or any other
suitable laboratory animal. The suckling rat is most pre-
ferred. An immune suppressant is any substance which will
impair the immune system of the animal to which it is admin-
istered and is selected from the group consisting of ste-
roids, anti-inflammatory agents, prostaglandins, cellular
immune suppressants, iron, silica, particles, beads, lipid
emulsions and any other effective immune suppressant. Pref-
erably, the immune suppressant is cyclosporin, dexamethasone,
triamcinolone, cortisone, prednisone, ibuprof n or any other

related compound or combination of compounds. More prefer-
ably the immune suppressant is a lipid emulsion, and the
lipid emulsion of choice is intralipid. When the pharmaceu-
tical composition is immunoglobulin, the assay measures the
clearance potential of the administered immunoglobulin.

Clearance is evaluated by determining whether the phar-
maceutical composition enhances clearance of the infectious
agent from the animal. This is typically determined from a
sample of biological fluid, such as blood, peritoneal fluid,
or cerebrospinal fluid. The infectious agent is cultured
from the biological fluid in a manner suitable for growth or
identification of the surviving infectious agent. From
samples of fluid taken over a period of time after treatment,
one skilled in the art can determine the effect of the phar-
maceutical composition on the ability of the animal to clear
the infectious agent. However, further data may be obtained
by measuring survival of animals administered the pharmaceu-
tical composition over a period of time, preferably a period
of days. Typically, both sets of data are utilized. Results
are considered positive if the pharmaceutical composition
enhances clearance or decreases mortality. In situations in
which there is enhanced organism clearance, but the test ani-
mals still perish, a positive result is still indicated.

Another embodiment of the present invention is isolated
immunoglobulin which is reactive in a first assay with a
preparation of a first Staphylococcus organism, and in a sec-
ond assay with a preparation of a second Staphylococcus or-
ganism. The first and second assays may be any immunological
assays and preferably are binding assays, opsonization as-
says, clearance assays, or any combination of these assays.
The first and second Staphylococcus organisms may be of dif-
ferent species, and are preferably S. epidermidis and S.
aureus. Alternatively, the first and second Staphylococcus
organisms may be of different serotypes, which are preferably
S. epidermidis type I and S. epidermidis type II. In either
case, it is most preferred that one of the preparations of
Staphylococcus organisms be Staphylococcus epid rmidis (Hay,

ATCC 55133).  The first and second preparations of a Staphy-
lococcus organism may b  any preparations of a Staphylococcus
organism including intact cells, cells fractionated by chemi-
cal or physical means, or cell extracts and is preferably a
whole-cell or cell surface extract.  It is preferred that one
preparation is from S. epidermidis (Hay, ATCC 55133).  A
preparation of a Staphylococcus organism is comprised of
polysaccharides, proteins, lipids and other bacterial cell
components.  It is preferred that the preparation is a
polysaccharide and protein preparation, i.e., a preparation
that predominantly contains mixtures or combinations of
polysaccharides, proteins and glycoproteins.    A suitable
preparation may be prepared by isolating a culture of bacte-
rial cells of Staphylococcus epidermidis (Hay, ATCC 55133),
suspending the isolated cells in a mixture comprised of a
solution of trichloroacetic acid, stirring the mixture at
approximately 4°C, centrifuging the mixture and saving the
resulting supernatant, combining the supernatant with an al-
cohol, preferably absolute ethanol, incubating the alcohol-
supernatant combination at approximately 4°C to precipitate a
preparation, and isolating the precipitated preparation.

Isolated immunoglobulin of the present invention may be
isolated from pooled or single units of blood, plasma, sera
or tissue, such as placenta, or from any immunoglobulin
preparation derived therefrom, such as intravenous im-
munoglobulin (IVIG).  Procedures for the isolation of im-
munoglobulin from these substances are well-known to those of
ordinary skill in the art.  Briefly, one method comprises the
steps of removal of all cells and cellular debris from the
fluid, and fractionation of the immunoglobulin portion of the
fluid by methods such as chromatography, precipitation, or
extraction.  Details of these procedures and others are de-
scribed in Protein Purification: Principles and Practice
(R.K. Scopes, Springer-Verlag, New York, 1987), which is
hereby specifically incorporated by reference by way of ex-
ample.

Isolated immunoglobulin may be one or more antibodies
of any isotype, including IgG, IgM, IgD, IgA, or IgE. Iso-
lated immunoglobulin includes polyclonal antibodies, most
preferably of the IgG fraction. Isolated immunoglobulin also
includes monoclonal antibodies, most preferably of the IgG
isotype. Procedures for the identification and isolation of
a particular fraction or isotype of antibody are well-known
in the subject art. Numerous methods, by way of example, are
disclosed in Current Protocols in Immunology (J.E. Coligan et
al., eds., John Wiley & Sons, New York, 1991), which is
hereby specifically incorporated by reference. The present
invention also includes methods for making these antibodies.

A method for making polyclonal antibodies for the
treatment of a Staphylococcus infection comprises the steps
of introducing a preparation of a Staphylococcus organism to
a mammal, removing serum from the mammal, and isolating
polyclonal antibodies which react in a first assay with a
preparation of a first Staphylococcus organism and in a sec-
ond assay with a preparation of a second Staphylococcus or-
ganism. The first and second assays may be any immunological
assays and preferably are binding assays, opsonization as-
says, clearance assays, or any combination of these assays.
The first and second preparations of a Staphylococcus organ-
ism may be any preparations of a Staphylococcus organism in-
cluding intact cells, cells fractionated by chemical or
physical means, or cell extracts and is preferably a whole-
cell or cell surface extract. The preparation of a Staphylo-
coccus organism introduced into a mammal may also be any
preparation of a Staphylococcus organism including intact
cells, cells fractionated by chemical or physical means, or
cell extracts and is preferably a whole-cell or cell surface
extract. It is preferred that the preparation is from S.
epidermidis (Hay, ATCC 55133). A preparation of a Staphylo-
coccus organism is comprised of polysaccharides, proteins,
lipids and oth r bacterial cell components. It is preferred

that the pr paration is a polysaccharide and protein prepara-
tion, i.e., a preparation that predominantly contains mix-
tures or combinations of polysaccharides, proteins and glyco-
proteins.    A suitable preparation may be prepared by iso-
lating a culture of bacterial cells of Staphylococcus
epidermidis (Hay, ATCC 55133), suspending the isolated cells
in a mixture comprised of a solution of trichloroacetic acid,
stirring the mixture at approximately 4°C, centrifuging the
mixture and saving the resulting supernatant, combining the
supernatant with an alcohol, preferably absolute ethanol,
incubating the alcohol-supernatant combination at ap-
proximately 4°C to precipitate a preparation, and isolating
the precipitated preparation.

     The Staphylococcus preparation introduced into a mammal
and used to make polyclonal antibodies may include specific
and nonspecific adjuvants.  Nonspecific adjuvants are sub-
stances which non-specifically stimulate the immune response
to an antigen and includes Freunds, water-oil emulsions, sur-
factants, mineral oils, synthetic polymers, aluminum hydrox-
ide, acrylamide, and other suitable response enhancing sub-
stances.  Specific adjuvants include specific T and B cell
stimulators which enhance the production of antibody by anti-
body producing cells.

     A method for making monoclonal antibodies for the
treatment of a Staphylococcus infection comprises creation of
hybridoma cells which produce monoclonal antibodies.  Such
procedures are well-known in the art.  Certain methods, by
way of example, are specifically described in Antibodies: A
Laboratory Manual (E. Harlow and D. Lane, Cold Spring Harbor
Lab., 1988), which is hereby incorporated by reference.  One
method comprises the isolation of antibody producing cells,
fusing the antibody producing cells with myeloma cells to
form hybridoma cells, and screening the resulting hybridoma
cells for a cell that produces the claimed monoclonal anti-
body.  The antibody producing cells isolated are selected
from the group consisting of cells which have been sensitized
by infection of a Staphylococcus organism in vivo, i.e., an

infection, cells which have been s nsitized by exposure to a
preparation of a Staphylococcus organism as herein d scribed,
in vivo, i.e., an immunization, cells which have been sensi-
tized by direct exposure of the cells in vitro, or cells
which have been sensitized by any other suitable means.  Iso-
lated antibody producing cells are fused with myeloma cells
using procedures which are well-known in the subject field.
A fusion procedure which employs polyethylene glycol or
Epstein-Barr virus is preferred.  The myeloma cell fusion
partners to the antibody producing cells are any cells which
are suitable for producing hybridoma cells.  This includes
myeloma cells which are of similar or dissimilar genetic ori-
gin.  By way of example, some suitable myeloma cell fusion
partners are the murine cell lines P3-X63Ag8, X63Ag.653, SP2/
0-Ag14, FO, NSI/1-Ag4-1, NSO/1, and FOX-NY, the rat cell
lines Y3-Ag1.2.3, YB2/0, and IR983F, and the human cell lines
U-266, FU-266, and HFB-1.  Hybridoma cells, immortalized by
fusion, are selected from the mixture of fused and unfused
cells using a suitable selection technique, such as
hypoxanthineguanine phosphoribosyl transferase (HGPRT) selec-
tion as disclosed in Monoclonal Antibodies: Principles and
Practice (J.W. Goding, Academic Press, San Diego, 1986),
which is hereby incorporated by reference for exemplary pur-
poses.  In an alternative method, antibody producing cells
may be immortalized using cytomegalovirus or another suitable
virus.  The resulting hybridoma cells, or cells produced us-
ing the alternate method, are screened for a cell that pro-
duces a monoclonal antibody which reacts in a first assay
with a preparation of a first Staphylococcus organism and in
a second assay with a preparation of a second Staphylococcus
organism.  The first and second assays may be any immunologi-
cal assays and preferably are binding assays, opsonization
assays, clearance assays, or any combination of these assays.
The first and second preparations of a Staphylococcus organ-
ism may be any preparations of a Staphylococcus organism in-
cluding intact c lls, cells fractionated by chemical or

physical means, or cell extracts and is pref rably a whole-
cell or cell surface extract. It is pref rr d that the first
and second preparations of a Staphylococcus organism are of
different serotype or species, and more preferred wherein the
first Staphylococcus organism is Staphylococcus epidermidis
(Hay, ATCC 55133).

The present invention also encompasses the DNA sequence
of the gene which codes for the isolated monoclonal antibody.
This DNA sequence can be identified, isolated, cloned, and
transferred to a prokaryotic cell or a eukaryotic cell for
expression by procedures which are all well-known in the sub-
ject field. Certain procedures, by way of example, are gen-
erally described in Current Protocols in Molecular Biology
(F.W. Ausubel et al., eds., John Wiley & Sons, 1989), which
is hereby specifically incorporated by reference.

It is preferred that monoclonal antibodies of the IgG
isotype are made, whether by direct isolation of an IgG pro-
ducing hybridoma cell or by genetic manipulation. One method
for the alteration of the isotype of the monoclonal antibody
involves the identification of the DNA sequence which codes
for the antigen binding site of the original antibody mol-
ecule. This DNA sequence is isolated or chemically synthe-
sized and cloned adjacent to the DNA sequence of the struc-
tural portion of a different immunoglobulin molecule which
may also be isolated or chemically synthesized. The result-
ing fusion product expressed from this clone would have the
antigen binding ability of the original antibody and the
structural portion of the new immunoglobulin gene chosen, in
other words, the new isotype.

Also preferred is the method wherein human monoclonal
antibodies are made. Purely human monoclonal antibodies are
made by the fusion of human antibody producing cells and hu-
man myeloma cells. Partly human monoclonal antibodies are
made by the utilization of nonhuman fusion partners to human
antibody producing cells or human myeloma cells. Nonhuman or
partly human antibodies may be mad more human by
chimerization wherein a nonhuman hybridoma cell is fused with

a human cell resulting in a hybridoma which is of dual or
triple (or more) genetic origin. Alternatively, a nonhuman
or a partly human antibody may be made more human by genetic
manipulation. Typically, this requires the cloning or the
chemical synthesis of DNA which encodes the amino acids of
the antigen binding site. This DNA sequence is cloned or
placed adjacent to the DNA sequence which codes for the
structural portion of a different antibody or amino acid se-
quence. In this way it is possible to change the overall
antigenic structure of the antibody molecule while retaining
the specific antigen binding ability. A murine antibody may
be altered to appear antigenically more human. This would be
very advantageous to reduce or eliminate a possible deleteri-
ous immune response. Furthermore, by placing the DNA se-
quence of the antigen binding site adjacent to the DNA se-
quence of another protein, the resulting fusion protein ex-
pressed is antigenically targeted. This could be especially
useful for targeting antibiotics, complement, or immunologi-
cal factors. The present invention includes an antigen bind-
ing site attached to the structural portion of an antibody
molecule or another protein which will react in a first assay
with a preparation of a first Staphylococcus organism and in
a second assay with a preparation of a second Staphylococcus
organism.

    Another embodiment of the present invention is a phar-
maceutical composition comprising isolated immunoglobulin as
herein described (including polyclonal antibodies and mono-
clonal antibodies), and a pharmaceutically acceptable car-
rier. Pharmaceutically acceptable carriers may be sterile
liquids, such as water and oils, including those of petro-
leum, animal, vegetable or of synthetic origin such as peanut
oil, soybean oil, mineral oil, sesame oil and the like. Wa-
ter is a preferred carrier when the pharmaceutical composi-
tion is administered intravenously. Saline solutions and
aqueous dextrose and glycerol solutions can also be employed
as liquid carriers, particularly for injectable solutions.
Suitable pharmaceutical carriers are described in <u>Remington's</u>

<u>Pharmaceutical Sciences, 18th Edition</u> (A. Gennaro, ed., Mack
Pub., Easton, Pa., 1990), which is hereby specifically incor-
porated by reference for exemplary purposes.

The invention also comprises a method of treating a
patient infected with or suspected of being infected with a
Staphylococcus organism comprising the administration of a
therapeutically effective amount of a pharmaceutical composi-
tion comprising immunoglobulin, polyclonal antibodies, or
monoclonal antibodies, and a pharmaceutically acceptable car-
rier.  A patient may be a human or an animal including the
dog, the cat, the cow, the sheep, the pig, the goat, and any
other suitable mammal, but is preferably a human.  Pharmaceu-
tically acceptable carriers are herein described.  A thera-
peutically acceptable amount is that amount of immunoglobulin
which is reasonably believed to provide some measure of re-
lief or assistance in the treatment of a Staphylococcus in-
fection.  Such therapy may be primary or supplemental to ad-
ditional treatment, such as antibiotic therapy, for a Staphy-
lococcus infection, an infection caused by a different agent,
or an unrelated disease.

A further embodiment of the present invention is a
method of preventing infection of a Staphylococcus organism
comprising the administration of a prophylactically effective
amount of a pharmaceutical composition, a passive vaccine,
comprising immunoglobulin, polyclonal antibodies, or mono-
clonal antibodies, and a pharmaceutically acceptable carrier,
all of which are herein described.  Treatment may be systemic
or localized.  Systemic treatment comprises administration of
the pharmaceutical composition by intravenous, intraperito-
neal, intracelial, intracorporeal injection, or any other
effective method of administration of a prophylactically ef-
fective amount.  Alternatively, the physiological composition
may be given locally.  This may also be by injection to the
particular area infected such as intramuscularly and also

subcutaneously. Localized treatment also comprises the ad-
ministration of a prophylactically effective amount of im-
munoglobulin by swabbing, immersing, soaking, or wiping, ei-
ther directly to a patient or to objects which are to be
placed within a patient, such as indwelling catheters, car-
diac values, cerebrospinal fluid shunts, joint prostheses,
other implants into the body, and any other objects, instru-
ments or appliances which carry a risk of becoming infected
with or introducing a Staphylococcus infection into a pa-
tient.

　　Another embodiment of the present invention is isolated
antigen. As used herein, isolated antigen means any single
antigen, any mixture of different antigens, or any combina-
tion of antigens which are separated from one or more differ-
ent organisms. Isolated antigen may be comprised of pro-
teins, polysaccharides, lipids, glycoproteins, or any other
suitably antigenic materials. Preferably, isolated antigen
contains proteins, polysaccharides and glycoproteins. Most
preferably, isolated antigen contains proteins and glycopro-
teins. It is also most preferred that isolated antigen be a
single purified antigen or a small number of purified anti-
gens which may be proteins, polysaccharides, glycoproteins,
or synthetic molecules. Methods of macromolecular purifica-
tion include filtration, fractionation, precipitation, chro-
matography, affinity chromatography, HPLC, FPLC, electro-
phoresis, and any other suitable separation technique. Meth-
ods for the purification of proteins are well-known in the
art. Preferably, isolated antigen is purified by a method
comprising the isolation of a culture of bacterial cells of
Staphylococcus, suspending the isolated cells in a mixture
comprised of a solution of trichloroacetic acid, stirring the
mixture at approximately 4°C, centrifuging the mixture and
saving the resulting supernatant, combining the supernatant
with an alcohol, incubating the alcohol-supernatant combina-
tion at approximately 4°C to precipitate antigen, and isolat-
ing the precipitated antigen. More preferred is a method
wherein the bacterial cells utilized are S. epidermidis (ATCC

55133). By way of example, a numb r of protein purification
m thods are d scribed in <u>Proteins: Structures and Mol cular
Properties</u> (T.E. Creighton, W.H. Freeman and Co., New York,
1984), which is hereby specifically incorporated by refer-
ence. Numerous methods for the purification of polysac-
charides are well-known in the art. By way of example, some
of these methods are described in <u>Carbohydrate Analysis: A
Practical Approach, 2nd Edition</u> (D. Rickwood, ed., IRL Press,
Oxford England, 1984), which is hereby specifically incorpo-
rated by reference. Methods for the identification, produc-
tion and use of synthetic antigens are also well-known in the
art. By way of example, a number of these methods are de-
scribed in <u>Laboratory Techniques in Biochemistry and Molecu-
lar Biology: Synthetic Polypeptides as Antigens</u> (R.H. Burden
and P.H. Knippenberg, eds., Elsevier, New York, 1988), which
is hereby specifically incorporated by reference.

Isolated antigen, upon introduction into a host, gener-
ates an antibody, which may be polyclonal or monoclonal,
which reacts in a first assay with a preparation of a first
Staphylococcus organism and in a second assay with a prepara-
tion of a second Staphylococcus organism. The first and sec-
ond preparations of a Staphylococcus organism may be any
preparations of a Staphylococcus organism including intact
cells, cells fractionated by chemical or physical means, or
cell extracts and is preferably a whole-cell or cell surface
extract. Preferably, the first and second Staphylococcus
organisms are of different serotypes or of different species.
It is also preferred that one preparation is from <u>S.
epidermidis</u> (Hay, ATCC 55133). A preparation of a Staphylo-
coccus organism is comprised of polysaccharides, proteins,
lipids and other bacterial cell components. It is preferred
that the preparation is a polysaccharide and protein prepara-
tion, i.e., a preparation that predominantly contains mix-
tures or combinations of polysaccharides, proteins and glyco-
proteins. A suitable preparation may be prepared by isolat-
ing a culture of bact rial cells of <u>Staphylococcus
epidermidis</u> (Hay, ATCC 55133), suspending th isolated cells

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in a mixture comprised of a solution of trichloroacetic acid,
stirring the mixture at approximately 4°C, centrifuging the
mixture and saving the resulting supernatant, combining the
supernatant with an alcohol, preferably absolute ethanol,
incubating the alcohol-supernatant combination at ap-
proximately 4°C to precipitate a preparation, and isolating
the precipitated preparation.

The first and second assays may be any immunological
assays and preferably are binding assays, opsonization as-
says, clearance assays, or any combination of these assays.
One preferred method employs a binding assay, which is herein
described, wherein isolated antigen generated antibody is
reacted in a binding assay with a preparation of a Staphylo-
coccus organism. The binding assay is preferably an ELISA,
or a RIA, but may also be an agglutination assay, a
coagglutination assay, a colorimetric assay, a fluorescent
binding assay, or any other suitable binding assay. It may
be performed by competitive or noncompetitive procedures with
results determined directly or indirectly.     Another pre-
ferred method employs an _in vitro_ opsonization assay which
may be a colorimetric assay, a chemilumenescent assay, a
fluorescent or radiolabel uptake assay, a cell mediated bac-
tericidal assay, or any other appropriate assay which mea-
sures the opsonic potential of a substance. A preferred
opsonization assay is the cell mediated bactericidal assay
which is herein described. The opsonization assay may use
antibody, which may be polyclonal or monoclonal, that has
been generated by isolated antigen. In this case, the assay
would measure the opsonic activity of the generated antibody,
thus providing an indirect determination of the opsonizing
potential of isolated antigen.

Another preferred method of identifying immunoglobulin
for the treatment of a Staphylococcus infection employs a
clearance assay. A preferred clearance assay is conducted in
an animal model which has been described herein. A particu-
larly useful animal model comprises the steps of administer-
ing a pharmaceutical composition, an immun  suppressant, and

a Staphylococcus organism to an immature animal, and evaluat-
ing whether the pharmaceutical composition reduces mortality
of the animal or enhances clearance of the Staphylococcus
organism from the animal. The pharmaceutical composition may
comprise isolated antigen or antigen generated antibody,
which may be polyclonal or monoclonal. When the pharmaceuti-
cal composition comprising isolated antigen is administered
to the animal, the assay measures the effect of isolated an-
tigen on the animal's own immune system. When the pharmaceu-
tical composition comprising the generated antibody is admin-
istered, the assay measures the effect of the administered
antibody. This assay may use any immature animal including
the rabbit, the guinea pig, the mouse, the rat, or any suit-
able laboratory animal. The suckling rat is most preferred.

Another preferred embodiment of the present invention
is a vaccine comprised of isolated antigen and a pharmaceuti-
cally acceptable carrier which, upon introduction into a
host, generates an antibody which is protective against in-
fection by a Staphylococcus organism. A pharmaceutically
acceptable carrier is herein described. Isolated antigen is
herein described and is any single antigen, any mixture of
different antigens, or any combination of antigens which are
separated from one or more different organisms. Vaccinations
would be particularly of benefit to those individuals who are
known to be or suspected of being at risk of Staphylococcus
infection. This includes patients who are preparing to un-
dergo surgery which involves breakage or damage of skin or
mucosal tissue, certain health care workers, and patients
whose immune systems are expected to become impaired from
some form of therapy such as chemotherapy or radiation
therapy for the treatment of cancer.

A further embodiment of this invention comprises a
method of treatment with this pharmaceutical composition. A
method of treating a human, or any animal, infected with or
suspected of being infected with a Staphylococcus organism,
compris s the administration of a therapeutically effective

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amount of the pharmac utical composition. A method of pre-
venting infection of a Staphylococcus organism in a human, or
any animal, comprises the administration of a prophylacti-
cally effective amount of the pharmaceutical composition. In
either situation, administration of the pharmaceutical compo-
sition may involve single or multiple doses given systemi-
cally to the entire individual. Administration may be by
injection, such as intravenous, intraperitoneal or subcutane-
ous. Methods for the therapeutic and prophylactic adminis-
tration of pharmaceutical compositions are well-known in the
art or may be determined with a reasonable degree of experi-
mentation. A number of examples are described in _The Pharma-_
_ceutical Basis of Therapeutics, 8th Edition_ (A.G. Goodman et
al., editors, Pergamon Press, New York, 1990), which is
hereby specifically incorporated by reference.

A still further embodiment of the present invention is
a method for evaluating the efficacy of a pharmaceutical com-
position used to treat an infectious agent comprising the
steps of administering the pharmaceutical composition, an
immune suppressant, and an infectious agent to an immature
animal, which is preferably a suckling rat, and evaluating
whether the pharmaceutical composition reduces mortality of
the animal or enhances clearance of the infectious agent from
the animal. This method may be used wherein the infectious
agent is selected from the group consisting of a bacterium,
preferably a gram positive bacterium, a parasite, a fungus
and a virus. An immune suppressant is any substance which
will impair the immune system of the animal to which it is
administered and is selected from the group consisting of
steroids, anti-inflammatory agents, prostaglandins, cellular
immune suppressants, iron, silica, particles, beads, lipid
emulsions and any other effective immune suppressant. Pref-
erably, the immune suppressant is cyclosporin, dexamethasone,
triamcinolone, cortisone, prednisone, ibuprofen or any other
related compound or combination of compounds. More prefer-
ably the immune suppressant is a lipid emulsion, and the
lipid emulsion of choice is intralipid. The pharmaceutical

composition is preferably administered prophylactically to
evaluate the  fficacy of the pharmaceutical composition in
enhancing resistance to an infectious agent, or therapeuti-
cally to evaluate the efficacy of the pharmaceutical composi-
tion in directly killing the infectious agent or enhancing
the immune response of an infected animal to fight off the
infection.

A still further embodiment of the present invention is
a diagnostic aid for the detection of a Staphylococcus infec-
tion and methods for the use of the diagnostic aid.  The di-
agnostic aid comprises immunoglobulin which may be polyclonal
or monoclonal antibodies, or isolated antigen, and a sample
of biological fluid containing or suspected of containing
antigen or antibody to Staphylococcus.  A method for the de-
tection of a Staphylococcus infection in an animal comprises
the addition of a biological sample containing or suspected
of containing antibody which is specific for Staphylococcus,
with isolated antigen, and determining the amount of binding
of the antibody to the isolated antigen.  Alternatively, this
method comprises a biological sample containing or suspected
of containing Staphylococcus antigen, and immunoglobulin
which is specific to a preparation of a Staphylococcus organ-
ism.  The immunoglobulin comprises polyclonal or monoclonal
antibody, but is preferably a monoclonal antibody.  Either
method may be an ELISA, a RIA, a colorimetric assay, an ag-
glutination assay, or any other suitable detection assay.  It
may be performed with competitive or noncompetitive assays
using direct or indirect detection procedures.  Examples of
such methods are disclosed in Immunology:  A Synthesis (E.S.
Golub, Sinauer Assocs., Inc., Sunderland, Ma., 1987), which
is hereby specifically incorporated by reference.

In a non-limiting embodiment, the diagnostic aid may be
used to identify pathogenic Staphylococcus.  Staphylococci
can be grouped into two groups based on a coagulase test
(Coagulase-negative, of which Staphylococcus epidermidis is
the most common pathogen, and coagulase-positive, of which

Staphylococcus aureus is the most common pathog n).  A labo-
ratory isolate can b  any organism isolated from, for ex-
ample, a human source, an animal source, or other source, by
microbiological techniques.  Laboratory isolates may also
contain non-pathogenic contaminants.  The diagnostic aid can
be used in the laboratory to determine if Staphylococci, par-
ticularly coagulase-negative Staphylococci, in the isolate
are pathogenic.  The methods described above for performing
the assays are applicable in this embodiment.  The diagnostic
aid can be used to identify Staphylococci, and antigens
thereof, in body fluids of an animal.  In a non-limiting em-
bodiment, a diagnostic aid capable of reacting with
coagulase-negative pathogenic Staphylococci is used to iden-
tify the presence of pathogenic Staphylococci, or antigens
thereof, in body fluids, which include but are not limited to
cerebrospinal fluid, blood, peritoneal fluid, and urine.  The
diagnostic aid is employed according to the methods described
above.  The detection using this diagnostic aid can be per-
formed in cases of actual or suspected acute or chronic in-
fection with Staphylococci.  Likewise, antigens from patho-
genic Staphylococcus organisms can be used to detect antibody
to pathogenic organisms in blood and body fluids.

A further object of the present invention is a method
for the detection of a pharmaceutical composition in a bio-
logical sample.  When the pharmaceutical composition com-
prises immunoglobulin, the method comprises the addition of a
biological sample containing the pharmaceutical composition
with isolated antigen, and determining the amount of binding
of the pharmaceutical composition to the isolated antigen.
Alternatively, when the pharmaceutical composition comprises
isolated antigen, this method comprises the addition of a
biological sample containing the pharmaceutical composition
with an antibody specific for the pharmaceutical composition,
and determining the amount of binding of the pharmaceutical
composition to the antibody.  These methods may be used, in-
ter alia, to determine the half-life, follow the route of
distribution and identify breakdown products of a particular

pharmaceutical composition. With this information, better
care can be provided by determining the best course of treat-
ment with that pharmaceutical composition.

Example 1

One object of the present invention is the identifica-
tion of immunoglobulin which is reactive in an assay with a
preparation of a first Staphylococcus organism and with a
preparation of a second Staphylococcus organism. IgG frac-
tions of standard intravenous immunoglobulin (IVIG) were used
in these experiments to represent large immunoglobulin pools.
Preparations of various pools of IgG from several companies
were analyzed for comparison (Gamimmune, Cutter Labs., Inc.,
Berkeley, California: Sandoglobuin, Sandoz, East Hanover,
N.J.; Gammagard, Hyland, Los Angeles, California; Polygam,
American Red Cross, Washington, D.C.).

Samples from each of these pools and one sample from an
individual patient (SAM), were tested for binding in an en-
zyme immune assay, specifically an enzyme-linked
immunosorbent assay (ELISA), against a preparation of S.
epidermidis. Although any S. epidermidis strain could be
used, these experiments used Hay, a clinical strain isolated
from the blood of a child with S. epidermidis sepsis. This
strain is on deposit at the American Type Culture Collection
(ATCC) and has been assigned number 55133. Briefly, a cul-
ture of S. epidermidis (Hay, ATCC 55133) was grown to log
phase (18-36 hours) at 37°C in 1600 ml aliquots of tryptic
soy broth (Difco Labs., Detroit, Mi.). The culture was cen-
trifuged at 5000 rpm for 10 minutes and the cell buttons
resuspended in a small volume (10-25 mls) of 2% trichloroace-
tic acid (TCA) at pH 2.0. The TCA suspensions were combined
and stirred overnight at 4°C. The next day, the combined
suspension was centrifuged at 5000 rpm for 10 minutes, the
supernatants aspirated and saved, and the cell buttons dis-
carded. Supernatants were combined with four volumes of ab-
solute ethanol and stored overnight at 4°C. This solution
was centrifuged at 2500 rpm for 10 minutes, the supernatants
aspirated and discarded, and the antigen precipitates

resuspended in saline and cultured to ensure sterility. Saline suspensions were lyophilized and stored at 4°C. TCA antigen for ELISA testing was made from each serotype by dissolving 1.0 mg of lyophilized extract in 40 mls of coating buffer. Coating buffer was prepared by combining 1.59 g $Na_2CO_3$, 2.93 g $NaHCO_3$, and 0.2 g $NaN_3$ and adding distilled water to a final volume of 1000 mls. This solution was adjusted to a pH of 9.6. One hundred microliter aliquots of the antigen-containing solution were added to each well of 96-well microtiter plates utilizing separate plates for each serotype. Plates were incubated overnight at 4°C, after which, wells were emptied and rinsed four times with PBS-Tween. PBS-Tween was prepared by combining 8.0 g NaCl, 0.2 g $KH_2PO_4$, 2.9 g $Na_2HPO_4$, 0.2 g KCl, 0.2 g $NaN_3$, and 0.5 mls of Tween-20 and adding distilled water to a final volume of 1000 mls. The solution was adjusted to a pH of 7.4. Samples of 100 uls from each pool of immunoglobulin were added to wells. Plates containing antisera were incubated at 4°C for two hours, after which, they were again emptied and rinsed four times with PBS-Tween. A 1/400 dilution of stock alkaline phosphatase-conjugated goat anti-rabbit IgG (Sigma Chem. Co., St. Louis, Mo.) was prepared in PBS-Tween. Aliquots of 40 uls were added to each well of the microtiter plates and the plates were incubated for two hours at 4°C. The plates were again emptied and rinsed four times with PBS-Tween. A 1 mg/ ml solution of p-nitrophenyl phosphate (Sigma Chem. Co., St. Louis, Mo.) was prepared in diethanolamine buffer and 100 ul aliquots of this solution were added to each well of the microtiter plates. Diethanolamine buffer was prepared by combining 97 mls diethanolamine and 0.2g $NaN_3$, and adding distilled water to a final volume of 1000 mls. The solution was adjusted to a pH of 9.8. These plates were incubated at 37°C for two hours. Absorbence was measured at 405 nm using the Multiskan® MCC/340 instrument (Flow Labs., Lugano, Switzerland).

## TABLE I

Antigen Binding Activity of Human Immunoglobulin for
Staphylococcus epidermidis (ATCC 55133)

| Immunoglobulin | Optical Density |
|---|---|
| 0609 | 0.707 |
| 163 | 0.731 |
| 0224 | 0.648 |
| 40R07 | 1.014 |
| 110 | 0.786 |
| 2801 | 0.666 |
| 40R09 | 1.026 |
| 069 | 0.901 |
| SAM | 1.002 |

As indicated in Table I, there was a marked difference
in the binding activity of each pool tested. Most samples
contained low levels of antibody to S. epidermidis. Inter-
estingly, a sample with one of the lowest activities (2801)
and the sample with the highest (40R09) are both from the
same source, Cutter Laboratories. Among the higher binding
pools, 069 and 40R09 were obtained from separate companies.
This data indicates that no single method of immunoglobulin
preparation can ensure the presence of a high titer of anti-
body to S. epidermidis, despite the fact that each of the
tested pools represent very large collections of human sera.
Variations in the content of reactive antibody occurred be-
tween preparations prepared by the same company and between
lots of the same preparation indicating that all im-
munoglobulin pools are distinct and that differences in the
content of a specific-identifiable antibody can be striking.
Example 2

In a second immunoglobulin binding study, random
samples of plasma from almost one hundred human patients were
screened in an ELISA. Antibody titers to four different
strains of S. epidermidis were determined. One strain was
obtained from the American Type Culture Collection,
Rockville, Maryland (ATCC 31423; Serotype I). Two others
(Serotypes II and III) were provided by Dr. Y. Ichiman of the
St. Marianna University School of Medicine, Japan, and have
been previously described (Y. Ichiman, J. Appl. Bacteriol.

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56:311 (1984)). Preparations of each were prepared as be-
fore. The ELISA was performed as previously described except
that 40 uls of each sample were used. As shown in Figure 1,
a significant number of samples contained antibody to each
strain of S. epidermidis including the clinical strain, Hay
(ATCC 55133). This data indicates that although there was a
great deal of variability in binding, there may be cross-
reacting antibodies within a single sample.

Example 3

To rule out the possibility that the samples of Figure
1 simply contained large numbers of distinct and strain-
specific antibodies to S. epidermidis, rabbits were immunized
with either a heat-killed whole cell or a TCA prepared vac-
cine of a preparation of S. epidermidis. TCA treated prepa-
rations of S. epidermidis were prepared as described. One
milligram of this preparation was dissolved in 1.0 ml of nor-
mal saline, and administered intramuscularly to New Zealand
White rabbits. Following a one week rest, a second 1.0 ml
dose was given. A final dose given one week later completed
the primary immunization series. An identical third (P3),
fourth (P4), or fifth (P5) course of immunization can be in-
cluded and additional booster series as above may be used to
further elevate specific antibody levels. Further booster
immunizations were given at additional intervals.

The whole bacterial cell vaccine was prepared as fol-
lows. Tryptic soy broth was inoculated with S. epidermidis
(Hay, ATCC 55133) and incubated for three hours at 37°C. A
20 ml aliquot of this preparation was centrifuged at 3000 rpm
for 10 minutes, the supernatant discarded, and the cell pel-
let resuspended in normal saline. A second washing with sa-
line was carried out following a repeat centrifugation and
the final suspension was prepared in saline so as to yield a
total volume of 10 mls. The bacteria were heated to 56°C for
60 minutes to produce the heat killed whole cell vaccine
which was cultured to ensure sterility. One milliliter
(about $10^9$ cells) of this whole cell preparation was adminis-
tered intrav nously to New Zealand White rabbits daily for

five days.  Aft r a one week rest, the rabbits were again
immunized daily for five days.  An identical third (P3),
fourth (P4), or fifth (P5) course of immunization can be in-
cluded and additional booster series as above may be used to
further elevate specific antibody levels.  Further booster
immunizations were given at additional intervals.

Sera obtained after immunization with the whole cell
preparation showed a marked increase in antibodies to S.
epidermidis, while the overall magnitude of the immune re-
sponse was reduced in serum obtained after TCA antigen im-
munization (Figures 2 and 3).  However, both the TCA treated
sera and the whole cell treated sera produced broadly reac-
tive antibodies to all three serotypes of S. epidermidis plus
the vaccine strain.  As there was only a single strain to
which these animals were originally exposed, and there was an
equivalent background level of binding before immunization,
it is clear that both preparations of S. epidermidis (Hay,
ATCC 55133) produced antibodies reactive with multiple S.
epidermidis serotypes.

Example 4

All antibodies, even those directed against a given
organism, may not enhance immunity and provide enhanced pro-
tection from infection.  Stated differently, antibodies which
bind to an antigen may not necessarily enhance opsonization
or clearance of that antigen from the infected animal.
Therefore, a neutrophil mediated bactericidal assay was used
to determine the functional activity of antibody to S.
epidermidis.  Neutrophils were isolated from adult venous
blood by dextran sedimentation and ficoll-hypaque density
centrifugation.  Washed neutrophils were added to round-
bottomed wells of microtiter plates (approximately $10^6$ cells
per well) with approximately $3x10^4$ mid-log phase bacteria (S.
epidermidis Hay, ATCC 55133).  Newborn rabbit serum (10 uls),
screened to assure absence of antibody to S. epidermidis, was
used as a source of active complement.  Forty microliters of
immunoglobulin (or serum) w re added at various dilutions and
the plates wer  incubated at 37°C with constant, vigorous

shaking.  Samples of 10 uls were taken from each well at zero
time and after 2 hours of incubation.  Each was diluted, vig-
orously vortexed to disperse the bacteria, and cultured on
blood agar plates overnight at 37°C to quantitate the number
of viable bacteria.  Controls consisted of neutrophils plus
complement alone.  Results are presented as percent reduction
in numbers of bacterial colonies observed compared to control
samples.

### TABLE II.

Opsonic Activity of Pools of Human Immunoglobulin
for Staphylococcus epidermidis (ATCC 55133)

| Immunoglobulin | Opsonic Activity (Percent) |
|---|---|
| 0609 | 18 |
| 163 | 8 |
| 0224 | 54 |
| 40R07 | 92 |
| 110 | 12 |
| 2801 | 45 |
| 40R09 | 90 |
| 069 | 15 |
| 2926 | 0 |
| 004 | 54 |
| 100 | 3 |
| 2807 | 23 |
| SAM | 97 |
| control* | 0 |

(* = neutrophil plus complement alone)

Opsonic activity varied from 0% to 23% with some
samples and from 90% to 97% with others.  As was observed in
the binding assay, no correlation could be drawn between pre-
parative techniques used and functional activity observed.
However, some of the immunoglobulin which had a high degree
of binding in Table I (O.D. > 1.0), also had a high level of
opsonic activity in Table II (e.g., 40R07, 40R09 and SAM).
In other words, only some of the immunoglobulin that bound to
TCA treated preparations of S. epidermidis promoted phagocy-
tosis and killing of S. epidermidis.  Thus, for the first
time using in vitro screening assays, one could select for
immunoglobulin which contains high levels of antibodies for

S. epidermidis that would also have reliable levels of anti-
body to prevent and treat S. epidermidis infections.

Example 5

It was important to determine if the opsonic antibodies
for S. epidermidis were specifically directed against sero-
type specific S. epidermidis antigens or if they were di-
rected against common staphylococcal antigens. To investi-
gate these alternatives, selected high-titer immunoglobulin
was preabsorbed with a preparation of S. epidermidis (Hay,
ATCC 55133) and tested for opsonic activity against three
different gram positive cocci. Absorbing bacteria were grown
overnight on blood agar plates, scraped from the plates, sus-
pended in normal saline, and pelleted in 0.5 ml microfuge
tubes to one-fifth the volume of the tube. After adding 0.4
mls of immunoglobulin to each, the tubes were vortexed and
rotated at a slow speed on an end-over-end tumbler (Fisher
Scientific Co., Pittsburgh, Pa.) at 4°C overnight. Bacteria
were sedimented the following day in a microfuge tube and the
supernatant was removed and filtered through a 0.2 um mem-
brane filter. The sterile immunoglobulin contained no de-
tectable S. epidermidis binding antibodies and was used ei-
ther directly or after storage at 70°C.

Selected high-titer immunoglobulin (directed im-
munoglobulin) showed opsonization of the two species of Sta-
phylococcus and the one species of Streptococcus tested (Fig-
ure 4). With selected immunoglobulin which has been
preabsorbed with a preparation of S. epidermidis, opsonic
activity to S. epidermidis was completely removed (95% to 0%
bactericidal activity). However, opsonic activity against
Streptococcus agalactiae, a different genus, was not dimin-
ished (93% to 94%). Surprisingly, opsonic activity of S.
aureus (kindly provided by Dr. Mendiola of the Walter Reed
Army Medical Center), which was present in the selected im-
munoglobulin at about half the level as antibody activity to
S. epidermidis, was r duced also suggesting that there are
antibodies to antigens shared by S. epidermidis and S.
aureus. Therefore, this selected immunoglobulin preparation

promoted opsonization by common anti-staphylococcal antibod-
ies that could be identified by absorption with S.
epidermidis. In the absence of antibody, there was no bacte-
ricidal activity demonstrated against any of the bacteria
(neutrophil plus complement alone). Thus, it can be con-
cluded that anti-staphylococcal antibodies were directed
against key staphylococcal antigens which could provide both
specific protection against S. epidermidis and broad protec-
tion against other Staphylococcus serotypes and species.

Example 6

Opsonic activity of serum from rabbits immunized with
the TCA prepared and the whole cell preparation was deter-
mined. Rabbits were immunized with either the TCA treated or
the whole cell preparation of S. epidermidis (Hay, ATCC
55133). Sera was collected as before and tested for opsoniz-
ing activity against three different serotype strains of S.
epidermidis plus the vaccine strain in the neutrophil medi-
ated bactericidal assay. As shown in Figures 5 and 6, both
the TCA treated and whole cell preparations induced an anti-
body response with very high opsonic activity against all
three serotypes. Although pre-vaccinated serum using the TCA
treated preparation did show some activity against serotype I
(Figure 5), opsonizing activity nearly doubled after inocula-
tion indicating that staphylococcal common antibodies were
indeed responsible. These data show that antibodies to S.
epidermidis capsular antigens are important for immunity and
that one or more antigens may be antigenically similar be-
tween different serotypes.

Example 7

The opsonizing activity of vaccinated rabbit sera was
again determined using S. aureus type 5 as the test bacterium
(Figure 7). Overall opsonizing activity against S. aureus
was not as high as activities observed against strains of S.
epidermidis, but serum samples from immunized animals did
provide significant activity compared to unvaccinated
samples. This data indicates that opsonizing antibodies to
S. epidermidis are also protective against S. aureus and

again suggests that theses antibodies may be directed against one or more staphylococcal common antigens.

Example 8

Many bacteria such as S. epidermidis are not pathogenic in normal humans. However, in infants with an immature immune system and in those individuals with an impaired immune system S. epidermidis can cause sepsis and even death. Therefore, in any animal model of sepsis it is critical to include these factors. It has been determined that by utilizing an animal with an immature immune system and subjecting that animal to immunological suppressant, the situation observed with septic human patients can be studied. The suckling rat model has proven most useful for these studies and is the preferred animal model. Normal baby rats injected with S. epidermidis become bacteremic within two hours and begin to slowly clear the infection shortly thereafter.

## TABLE III.

### Staphylococcus epidermidis Bacteremia Levels in Suckling Rats Treated with Normal Saline

| Time Post Infection | Number Bacteremic | Percent Bacteremic | Bacteremia Level |
|---|---|---|---|
| 2 hours | 8/8 | 100 | $3.8 \times 10^2$ |
| 4 hours | 7/8 | 87.5 | $1.3 \times 10^2$ |
| 6 hours | 8/8 | 100 | $7.5 \times 10^2$ |
| 14 hours | 6/8 | 75 | $8.8 \times 10^1$ |
| 18 hours | 3/8 | 37.5 | $0.5 \times 10^1$ |
| 22 hours | 0/8 | 0 | 0 |

All of the animals cleared bacteremia within 72 hours after infection (Table III), suggesting that under normal circumstances, neonatal immunity, while impaired, can eventually control S. epidermidis. However, some studies in rats infected with S. epidermidis shortly after birth have demonstrated that a lethal infection can still develop (data not shown).

Example 9

The effect of intralipid on S. epidermidis mortality in suckling rats was assayed. Wistar rats were injected with intralipid, an immune suppressant, just after birth. Animals

were administered intralipid beginning on day two of life.
Two doses were administered each day for two days.  With the
final dose of intralipid, animals were also given selected
immunoglobulin or saline.  After this final dose the animals
were infected by subcutaneous injection with a preparation of
S. epidermidis (Hay, ATCC 55133).  Blood samples were subcul-
tured onto plates to ensure that bacteremia was caused by
Staphylococcus and to follow clearance after therapy.  All
animals were followed for five days to determine survival.

### TABLE IV.

Animal Model:  The Effect of Intralipid Dose on
Staphylococcus epidermidis Mortality in Suckling Rats

| | Survival | | | |
|---|---|---|---|---|
| Intralipid Dose | Infected | | Control | |
| 4 gm/kg | 10/10 | 100% | 7/7 | 100% |
| 8 gm/kg | 10/13 | 76% | 9/9 | 100% |
| 12 gm/kg | 7/12 | 58% | 11/11 | 100% |
| 16 gm/kg | 6/13 | 46% | 11/11 | 100% |
| *16 gm/kg | 2/6 | 33% | 5/5 | 100% |

* = Intralipid dose started on day one of life with infection
after final dose on day two.

Animals receiving only S. epidermidis successfully
overcame infection and survived.  Only those animals which
were treated with intralipid prior to infection showed a
marked decrease in their ability to resist S. epidermidis.
Death occurred with an increased frequency which correlated
with an increased dose of intralipid.

Example 10

The effectiveness of selected high-titer (directed)
immunoglobulin in providing protection against a lethal in-
fection of S. epidermidis (Hay, ATCC 55133) was determined in
the suckling rat model.  Two day old Wistar rats were given
two, 0.2 ml intraperitoneal injections of 20% intralipid.
The next day, animals were again given the same series of
injections of 20% intralipid plus immunoglobulin or serum
from vaccinated animals.  After the last injection, ap-
proximately $5 \times 10^7$ cells of S. epidermidis (Hay, ATCC 55133)

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were injected subcutaneously at the base of the tail.  Mor-
tality was determined for five days.

<div align="center">TABLE V.</div>

<div align="center">Effectiveness of Immunoglobulin Directed Against
<u>Staphylococcus epidermidis</u> in Providing Protection from
Lethal Infection</div>

| Immunoglobulin | Treated | Died | Mortality |
|---|---|---|---|
| Exp. #1 | | | |
| 40R09 | 24 | 0 | 0 % |
| Standard | 20 | 4 | 20 % |
| Control-untreated | 13 | 7 | 54 % |
|         -uninfected | 11 | 0 | 0 % |
| Exp. #2 | | | |
| 40R09 | 13 | 2 | 8% |
| Vaccine Induced | 11 | 2 | 18% |
| Control - saline | 19 | 11 | 42% |

Directed immunoglobulin, selected for the ability to
bind to or opsonize a preparation of S. epidermidis (lot No.
40R09), provided complete protection from lethal infection in
an immunity impaired animal model.  These results are identi-
cal to the results obtained from uninfected animals.
Unselected low-titer immunoglobulin (also called standard
immunoglobulin) demonstrated 20% mortality and other controls
were as expected.  Untreated and uninfected animals had
greater than 50% mortality.  In a second, similar experiment,
directed high-titer human immunoglobulin and vaccine induced
high-titer rabbit serum, both strongly protective, produced
nearly identical results, whereas a saline control had over
40% mortality.  Overall, these data suggest that it is indeed
the antibodies directed against S. epidermidis which are pro-
tective in the suckling rat model.

Example 11

Immunoglobulin which bound to a preparation of S.
epidermidis in an ELISA assay and opsonized S. epidermidis
organisms in the cell mediated bactericidal assay (directed
immunoglobulin) was tested for its capacity to promote clear-
ance of S. epidermidis in the suckling rat model.  Blood

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samples were taken from infected animals at regular intervals
(Figure 8).  Only directed immunoglobulin which had been pre-
viously identified in an ELISA or an opsonic assay decreased
levels of bacteria over the course of treatment and it was
these animals that showed increased survivals in Table V.
Immunoglobulin which did not opsonize or bind to a prepara-
tion of S. epidermidis did not promote clearance of bacteria
from the blood of infected animals.

Example 12

    Antibody to S. epidermidis was analyzed for the ability
to provide protection against an international geographically
diverse group of S. epidermidis strains in the suckling rat
clearance assay (Figure 9).  Directed immunoglobulin enhanced
survival against a clinical isolate from the United States
(ATCC 55133), a prototype laboratory strain (ATCC 31423, cap-
sular serotype I) and two distinct Japanese strains (capsular
serotypes II and III).  Directed immunoglobulin preabsorbed
against a preparation of S. epidermidis showed no increase in
survival (Figure 10).  Bacterial counts from blood samples
taken during the course of this study also showed that di-
rected immunoglobulin rapidly cleared Staphylococcus bacter-
emia.  Rats treated with saline or preabsorbed immunoglobulin
had persistent bacteremia and increased mortality (Figure
11).

    To determine if survival was related to functional
anti-Staphylococcus activity of antibody, immunoglobulin
preparations with various levels of opsonophagocytic bacteri-
cidal activity for S. epidermidis (directed immunoglobulin)
were compared with saline and preabsorbed immunoglobulin
(which had no bactericidal activity for S. epidermidis).  A
significant relationship was observed between
opsonophagocytic bactericidal activity of antibody and sur-
vival in Staphylococcus sepsis (Figure 12).  While saline,
standard immunoglobulin, and preabsorbed directed im-
munoglobulin provided similarly poor protection (each had
little or no opsonophagocytic bactericidal antibody), the
unabsorbed directed immunoglobulin provided uniformly good

survival indicating that the opsonic anti-Staphylococcus an-
tibodies present were associated with survival.

Example 13

Previous reports have suggested that there are multiple
S. epidermidis serotypes. In addition there are many other
coagulase negative staphylococci besides S. epidermidis. For
broadly reactive antibody to be most efficacious, it should
ideally cover all pathogenic coagulase negative
staphylococci. Many coagulase negative staphylococci,
however, rarely if ever cause infections in humans. Thus it
would be important to know if broadly reactive antibodies
would be able to bind to all human pathogenic coagulase
negative bacteria.

Rabbits were immunized with Staphylococci of one of
three S. epidermidis strains (ATCC-31432, S. epidermidis 360
and S. epidermidis 10). S. epidermidis 360 is of the same
serotype as the type strain, Hay (ATCC 55133). The antisera
were identified as follows: Anti-I was raised against strain
ATCC 31432; Anti-II was raised against strain S. epidermidis
360; and Anti-III was raised against strain S. epidermidis
10.

Coagulase negative staphylococci isolated from patients
were speciated and characterized as pathogens if in a given
patient there were $\geq 2$ positive cultures from normally sterile
sites (cultures obtained at different times or from different
sites). These cultures were then reacted with rabbit
antisera (Anti-I, Anti-II and Anti-III) in an ELISA assay.

ELISA Assay

*Preparation of ELISA plates.* 100 λ aliquots of S.
epidermidis extracted antigens are added to wells of 96 well
microassay plates (Nunclon®, Nunc, Denmark), and these are
stored overnight at 4°C. Wells are gently washed with Tween
(0.5 ml Tween 20/1 deionized $H_2O$) prior to use.

*Preparation of antisera.* Rabbit antisera designated
anti-I, anti II, and anti-III sera were produc d according to
the general method of Fischer G.W. et al., J. Exp r. Med.
148:776-786 (1978). Antiserum preparation are then diluted

100 fold in PBS-Tween prior to use. Further serial dilutions
are likewise carried out in PBS-Tween. The rabbit antisera
(anti-I, anti-II, anti-III) were prepared further by
absorption with the two heterologous strains to remove common
staphylococcal antibodies not specific to one of the strains.

*Analysis of antibody reactivity.* Microassay plates
were prepared using 40$\lambda$ of antisera at several dilutions (1/
100-1/12800). Antisera was added to the appropriate wells of
the microassay plate. Normal saline was used as a control,
and was similarly diluted. Plates were incubated at 4°C for
two hours. Alkaline phosphatase-conjugated goat anti-rabbit
IgG (Sigma, St. Louis, MO) was prepared in a 1/400 dilution
with PBS-Tween. 40$\lambda$ of this preparation was then added to
each well in appropriate columns. PBS-Tween alone was added
to a single column of wells. Plates were again incubated at
4°C for two hours. 4-nitrophenyl phosphate was used as
substrate for the enzymatic reaction, and was prepared by
dissolving a 5 mg substrate tablet (104 phosphate substrate
tablets, Sigma) in 5 ml of 10% diethanolamine buffer (see
below). 100 $\lambda$ of this substrate preparation was then added
to each well as appropriate after incubation at 37°C, and
absorbance was then measured at 405 nm at 120 minutes using
the Titertek® Multiskan MCC/340 instrument (Flow
Laboratories, Lugano, Switzerland).

*Preparation of reagents.* Preparation of buffers from
the methods of Voller, D. et al, Bull. W.H.O. 53:55-64
(1976).

1.      Coating buffer (pH 9.6)

| | | |
|---|---|---|
| $Na_2CO_3$ | 1.59 g | |
| $NaHCO_3$ | 2.93 g | |
| $NaN_3$ | 0.2 g | |
| $H_2O$ | 1000 ml | |

2.    PBS-Tween (pH 7.4)

| | | |
|---|---|---|
| NaCl | 8.0 | g |
| $KH_2PO_4$ | 0.2 | g |
| $Na_2HPO_4$ | 2.9 | g |
| KCl | 0.2 | g |
| Tween 20 | 0.5 | ml |
| $NaN_3$ | 0.2 | |
| $H_2O$ | 1000 | ml |

3.    Diethanolamine buffer (pH 9.8)

| | |
|---|---|
| Diethanolamine | 97 ml |
| $NaN_3$ | 0.2 g |
| $H_2O$ | to 1000 ml |

The results of these studies are shown in Table VI.
There were three coagulase negative staphylococci besides S.
epidermidis that were identified as human pathogens. Each of
these pathogenic staphylococci reacted with rabbit antisera
obtained after immunization with a single S. epidermidis
strain (S. epidermidis 360). Absorbing this antiserum with
the other two S. epidermidis strains (used to produce the
other antisera but not this antisera) did not remove the
staphylococcal-reactive antibodies induced by this single S.
epidermidis strain. The antisera raised against the other
strains, however, did not react to any of the pathogenic
strains after absorption with S. epidermidis 360. In
addition, S. epidermidis Hay (ATCC 55133) reacted with the
broadly reactive antisera further showing that the antigens
from this organism bind the antibodies in the broadly
reactive antisera.

Ichiman and Yoshida divided S. epidermidis into 3
serotypes (J. Appl. Bacteriol. 51:229 (1981)). However, they
could not demonstrate that pathogenicity was associated with
any specific strain or strains using mouse virulence testing
(Ichiman, J. Appl. Bacteriol. 56:311 (1984)). The results
presented in this example demonstrate that all of the
pathogenic human coagulase negative staphylococci reacted
with the antibodi s elicited by immunization with a single S.

<u>epidermidis</u> strain, but not two other strains is a new
observation.

The immunizing <u>S</u>. <u>epidermidis</u> strain and <u>S</u>. <u>epidermidis</u>
Hay (ATCC 55133) both react with the antisera to which all of
the human pathogens reacted. The results demonstrate clearly
that antigens on the surface of the human pathogens and the
immunizing strain and <u>S</u>. <u>epidermidis</u> Hay (ATCC 55133) are
similar. Thus, antibodies to a single <u>S</u>. <u>epidermidis</u> with
the proper constituents (such as <u>S</u>. <u>epidermidis</u> Hay (ATCC
55133)) could confer broad protection against coagulase
negative staphylococci. In addition, antibodies raised
against these antigenic determinants are useful for
distinguishing between pathogenic and non-pathogenic
Staphylococci in laboratory isolates. The antibodies can
also be used to detect pathogenic Staphylococci, and antigens
thereof, or antibodies directed thereto, in mammalian body
fluids such as cerebrospinal fluid, blood, peritoneal fluid
and urine.

## TABLE VI

**Coagulase Negative Staphylococci
that are Human Pathogens\*
React with Antibodies Derived from Immunization
with a Single Coagulase Negative Staphylococcus**

| Organism | No. Isolated | Positive Reaction |
|---|---|---|
| S. epidermidis | 16 (57%) | 16/16 (100%) |
| S. haemolyticus | 8 (29%) | 8/8 (100%) |
| S. hominis | 3 (11%) | 3/3 (100%) |
| S. simulans | 1 (3%) | 1/1 (100%) |
| S. warneri | 0 | - |
| S. capitis | 0 | - |

\*Isolates were selected only from patients with $\geq 2$ positive cultures from sterile sites (different times or different sources).

Other embodiments and uses of the invention will be apparent to those skilled in the art from consideration of the specification and practice of the invention disclosed herein. It is intended that the specification and examples be considered exemplary only, with the true scope and spirit of the invention being indicated by the following claims.

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I Claim:

1.     A method of identifying immunoglobulin for the treatment of a Staphylococcus infection, comprising the steps of:

    a)     performing a first assay to identify immunoglobulin which is reactive with a preparation of a first Staphylococcus organism,

    b)     performing a second assay to identify immunoglobulin which is reactive with a preparation of a second Staphylococcus organism, and

    c)     selecting immunoglobulin which is reactive with the preparations from both the first and second Staphylococcus organisms.

2.     A method as claimed in claim 1 wherein each assay is a binding assay.

3.     A method as claimed in claim 2 wherein the first Staphylococcus organism is <u>Staphylococcus epidermidis</u> (ATCC 55133).

4.     A method as claimed in claim 3 wherein the preparation of <u>Staphylococcus epidermidis</u> (ATCC 55133) is prepared by a method comprising the steps of:

    a)     isolating a culture of bacterial cells of <u>Staphylococcus epidermidis</u> (ATCC 55133),

    b)     suspending the isolated cells in a mixture comprised of a solution of trichloroacetic acid,

    c)     stirring the mixture at approximately 4°C,

    d)     centrifuging the mixture and saving the resulting supernatant,

    e)     combining the supernatant with an alcohol,

    f)     incubating the alcohol-supernatant combination at approximately 4°C to precipitate a preparation, and

    g)     isolating the precipitated preparation.

5.　　A method as claimed in claim 1 wher in each assay is an opsonization assay.

6.　　A method as claimed in claim 5 wherein the opsonization assay is a cell mediated bactericidal assay.

7.　　A method as claimed in claim 1 wherein each assay is a clearance assay.

8.　　A method as claimed in claim 7 wherein the clearance assay comprises the steps of:

　　　　a)　　administering the immunoglobulin, an immune suppressant, and a Staphylococcus organism to an immature animal, and

　　　　b)　　evaluating whether the immunoglobulin reduces mortality of the animal or enhances clearance of the Staphylococcus organism from the animal.

9.　　A method as claimed in claim 1 wherein the first and second Staphylococcus organisms are of different serotypes.

10.　　A method as claimed in claim 1 wherein the first and second Staphylococcus organisms are of different species.

11.　　Isolated immunoglobulin which reacts in a first assay with a preparation of a first Staphylococcus organism and in a second assay with a preparation of a second Staphylococcus organism.

12.　　Isolated immunoglobulin as claimed in claim 11 wherein each assay is a binding assay.

13.　　Isolated immunoglobulin as claimed in claim 12 wherein the first Staphylococcus organism is Staphylococcus epidermidis (ATCC 55133).

14.　　Isolated immunoglobulin as claimed in claim 13 wherein the preparation of Staphylococcus epidermidis (ATCC 55133) is prepared by a method comprising the steps of:

　　　　a)　　isolating a culture of bacterial cells of Staphylococcus epidermidis (ATCC 55133),

　　　　b)　　suspending the isolated cells in a mixture comprised of a solution of trichloroacetic acid,

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c)   stirring the mixture at approximately 4°C,
     centrifuging the mixture and saving the
     resulting supernatant,

d)   combining the supernatant with an alcohol,

e)   incubating the alcohol-supernatant
     combination at approximately 4°C to
     precipitate a preparation, and

f)   isolating the precipitated preparation.

15.  Isolated immunoglobulin as claimed in claim 11
wherein each assay is an opsonization assay.

16.  Isolated immunoglobulin as claimed in claim 15
wherein the opsonization assay is a cell mediated
bactericidal assay.

17.  Isolated immunoglobulin as claimed in claim 11
wherein each assay is a clearance assay.

18.  Isolated immunoglobulin as claimed in claim 17
wherein the clearance assay comprises the steps of:

a)   administering the immunoglobulin, an immune
     suppressant, and a Staphylococcus organism
     to an immature animal, and

b)   evaluating whether the immunoglobulin
     reduces mortality of the animal or enhances
     clearance of the Staphylococcus organism
     from the animal.

19.  Isolated immunoglobulin as claimed in claim 11
wherein the first and second Staphylococcus organisms are of
different serotypes.

20.  Isolated immunoglobulin as claimed in claim 11
wherein the first and second Staphylococcus organisms are of
different species.

21.  Isolated immunoglobulin as claimed in claim 11
which comprises polyclonal antibodies.

22.  Isolated immunoglobulin as claimed in claim 21
which comprises an IgG fraction of polyclonal antibodies.

23.  Isolated immunoglobulin as claimed in claim 11
which comprises monoclonal antibodies.

24.    Isolat d immunoglobulin as claimed in claim 23 which comprises monoclonal antibodies of the IgG isotype.

25. Isolated immunoglobulin as claimed in claim 11 wherein said immunoglobulin reacts with pathogenic Staphylococcus organisms of different species.

26. Isolated immunoglobulin as claimed in claim 25 wherein said Staphylococcus organisms are coagulase-negative.

27.    A pharmaceutical composition comprising:

a)    isolated immunoglobulin as claimed in claim 11, and

b)    a pharmaceutically acceptable carrier.

28.    A method of treating a human infected with or suspected of being infected with a Staphylococcus organism comprising the administration of a therapeutically effective amount of the pharmaceutical composition of claim 27.

29.    A method of preventing infection of a Staphylococcus_organism in a human comprising the administration of a prophylactically effective amount of the pharmaceutical composition of claim 27.

30.    A method of making polyclonal antibodies for the treatment of a Staphylococcus infection, comprising the steps of:

a)    introducing a preparation of a Staphylococcus organism to a mammal,

b)    removing serum from the mammal, and

c)    isolating polyclonal antibodies which react in a first assay with a preparation of a first Staphylococcus organism and in a second assay with a preparation of a second Staphylococcus organism.

31.    A method of making monoclonal antibodies for the treatment of a Staphylococcus infection, comprising the steps of:

a)    isolating antibody producing cells,

b)    fusing the antibody producing cells with myeloma cells to form hybridoma cells, and

    c)      screening the resulting hybridoma cells for
a cell that produc s a monoclonal antibody
which reacts in a first assay with a
preparation of a first Staphylococcus
organism and in a second with a preparation
of a second Staphylococcus organism.

32.    Isolated antigen which, upon introduction into a
host, generates an antibody which reacts in a first assay
with a preparation of a first Staphylococcus organism and in
a second assay with a preparation of a second Staphylococcus
organism.

33.    Isolated antigen as claimed in claim 32 which is
isolated by a method comprising the steps of:

    a)      isolating a culture of bacterial cells of
Staphylococcus,

    b)      suspending the isolated cells in a mixture
comprised of a solution of trichloroacetic
acid,

    c)      stirring the mixture at approximately 4°C,

    d)      centrifuging the mixture and saving the
resulting supernatant,

    e)      combining the supernatant with an alcohol,

    f)      incubating the alcohol-supernatant
combination at approximately 4°C to
precipitate antigen, and

    g)      isolating the precipitated antigen.

34.    Isolated antigen as claimed in claim 32 wherein
the bacterial cells are Staphylococcus epidermidis (ATCC
55133).

35.    Isolated antigen as claimed in claim 32 wherein
each assay is a binding assay.

36.    Isolated antigen as claimed in claim 35 wherein
the first Staphylococcus organism is Staphylococcus
epidermidis (ATCC 55133).

37.    Isolated antigen as claimed in claim 32 wherein
each assay is an opsonization assay.

38.    Isolated antig n as claimed in claim 37 wherein
the opsonization assay is a cell mediated bactericidal assay.

39.    Isolated antigen as claimed in claim 32 wherein
each assay is a clearance assay.

40.    Isolated antigen as claimed in claim 39 wherein
the clearance assay comprises the steps of:

      a)    administering the antibody, an immune
          suppressant, and a Staphylococcus organism
          to an immature animal, and

      b)    evaluating whether the antibody reduces
          mortality of the animal or enhances
          clearance of the Staphylococcus organism
          from the animal.

41.    Isolated antigen as claimed in claim 32 wherein
the first and second Staphylococcus organisms are of
different serotypes.

42.    Isolated antigen as claimed in claim 32 wherein
the first and second Staphylococcus organisms are of
different species.

43.    A pharmaceutical composition comprising:

      a)    isolated antigen as claimed in claim 32,
and

      b)    a pharmaceutically acceptable carrier.

44.    A method of treating a human infected with or
suspected of being infected with a Staphylococcus organism
comprising the administration of a therapeutically effective
amount of the pharmaceutical composition of claim 43.

45.    A method of preventing infection with a
Staphylococcus_organism in a human comprising the
administration of a prophylactically effective amount of the
pharmaceutical composition of claim 43.

46.    A method of isolating antigen comprising the
steps of:

      a)    isolating a culture of bacterial cells of a
          Staphylococcus organism,

b)    suspending the isolated cells in a mixture
      comprised of a solution of trichloroacetic
      acid,

c)    stirring the mixture at approximately 4°C,

d)    centrifuging the mixture and saving the
      resulting supernatant,

e)    combining the supernatant with an alcohol,

f)    incubating the alcohol-supernatant
      combination at approximately 4°C to
      precipitate antigen, and

g)    isolating the precipitated antigen.

47.    A method of isolating antigen as claimed in claim
46 wherein the Staphylococcus organism is Staphylococcus
epidermidis (ATCC 55133).

48.    A method of evaluating the efficacy of a
pharmaceutical composition to treat an infectious agent
comprising the steps of:

a)    administering the pharmaceutical
      composition, an immune suppressant, and an
      infectious agent to an immature animal, and

b)    evaluating whether the pharmaceutical
      composition reduces mortality of the animal
      or enhances clearance of the infectious
      agent from the animal.

49.    A method as claimed in claim 48 wherein the
immature animal is a suckling rat.

50.    A method as claimed in claim 48 wherein the
immune suppressant is selected from the group consisting of
steroids, anti-inflammatory agents, prostaglandins, cellular
immune suppressants, iron, silica, particles, beads, and
lipid emulsions.

51.    A method as claimed in claim 48 wherein the
infectious agent is selected from the group consisting of a
bacterium, a parasite, a fungus and a virus.

52.    A method as claimed in claim 48 wherein the
infectious agent is a gram positive bacteria.

53.    A method as claimed in claim 52 wherein the gram positive bacteria is <u>Staphylococcus epidermidis</u>.

54.    A method as claimed in claim 48 wherein the pharmaceutical composition is administered prophylactically.

55.    A method as claimed in claim 48 wherein the pharmaceutical composition is administered therapeutically.

56.    A diagnostic aid for the detection of a Staphylococcus infection comprising a biological sample containing or suspected of containing Staphylococcus antigen and isolated immunoglobulin as claimed in claim 11.

57.    A diagnostic aid for the detection of a Staphylococcus infection comprising a biological sample containing or suspected of containing antibody to Staphylococcus and isolated antigen as claimed in claim 32.

58.    A method for the detection of a Staphylococcus infection comprising the addition of a biological sample containing or suspected of containing Staphylococcus antigen to isolated immunoglobulin as claimed in claim 11, and determining the amount of binding of the Staphylococcus antigen to the isolated immunoglobulin.

59.    A method for the detection of a Staphylococcus infection comprising the addition of a biological sample containing or suspected of containing antibody which is specific for Staphylococcus to isolated antigen as claimed in claim 32, and determining the amount of binding of the antibody to the isolated antigen.

60.    A method for the detection of a pharmaceutical composition in a biological sample comprising the addition of the biological sample containing the pharmaceutical composition to isolated immunoglobulin as claimed in claim 11, and determining the amount of binding of the pharmaceutical composition to the isolated immunoglobulin.

61.    A method for the detection of a pharmaceutical composition in a biological sample comprising the addition of the biological sample containing the pharmaceutical composition to isolated antigen as claimed in claim 32, and

determining the amount of binding of the pharmaceutical
composition to the isolated antigen.

62. A method of identifying pathogenic Staphylococcus
in a laboratory isolate, said method comprising reacting said
laboratory isolate with the isolated immunoglobulin of claim
25 in an assay.

63. The method of claim 62, wherein said assay is a
binding assay.

64. The method of claim 62, wherein said assay is
selected from the group consisting of an ELISA assay and a
radioimmunoassay.

65. A method of detecting antibodies to pathogenic
Staphylococci, said method comprising reacting said
antibodies with the antigen of claim 32 in an assay, wherein
said first and second Staphylococcus organisms are
pathogenic.

66. A method of detecting antigens from pathogenic
Staphylococci, said method comprising reacting said antigens
with the isolated immunoglobulin of claim 25 in an assay.

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Figure 1

Figure 2

Figure 3

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Figure 4

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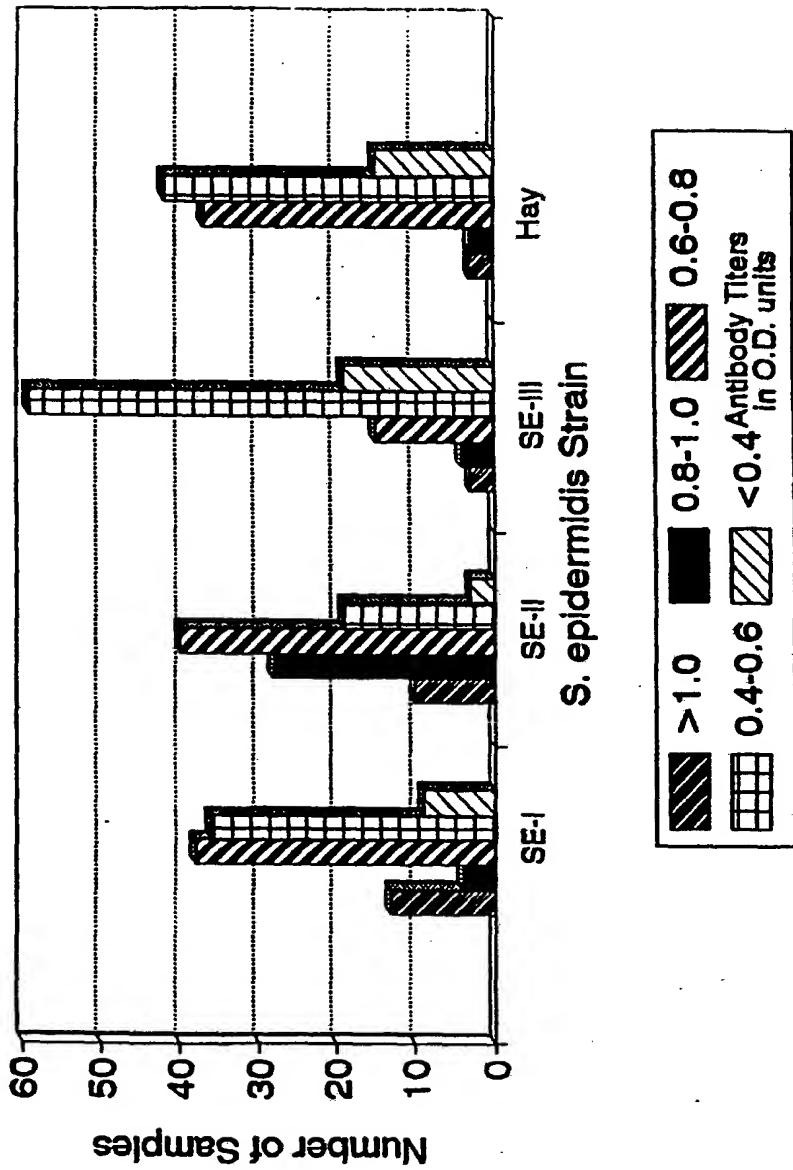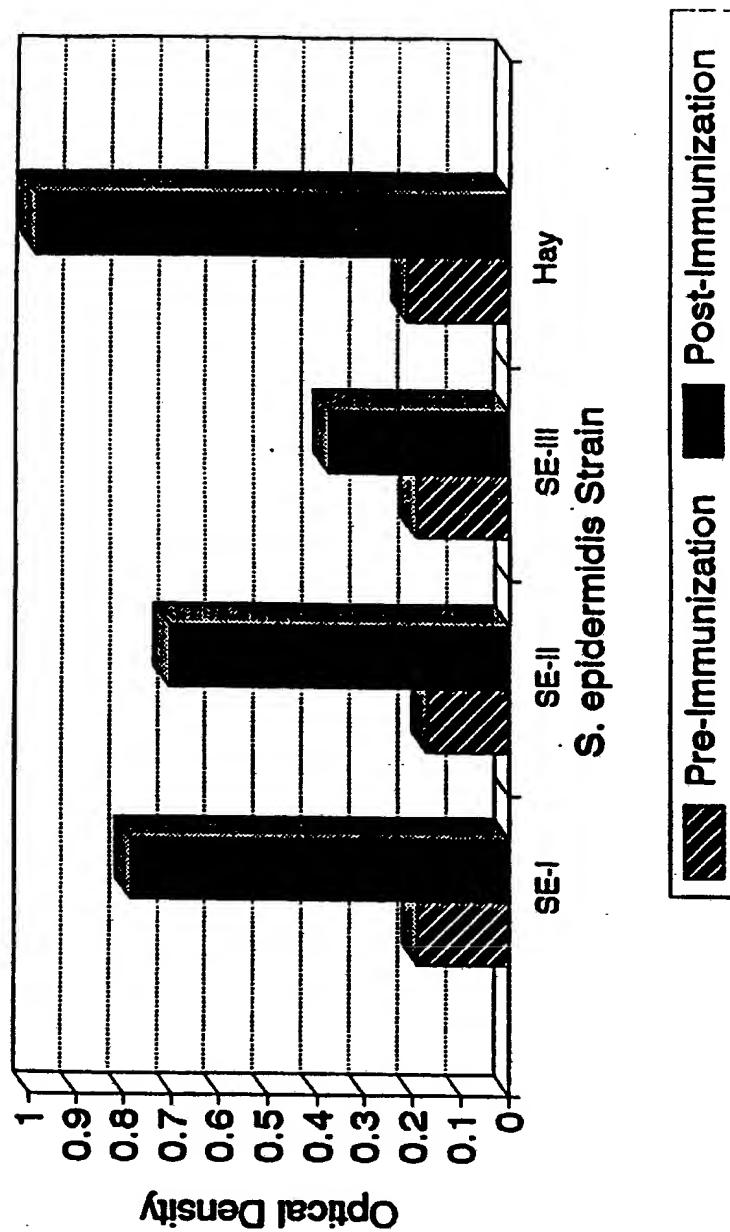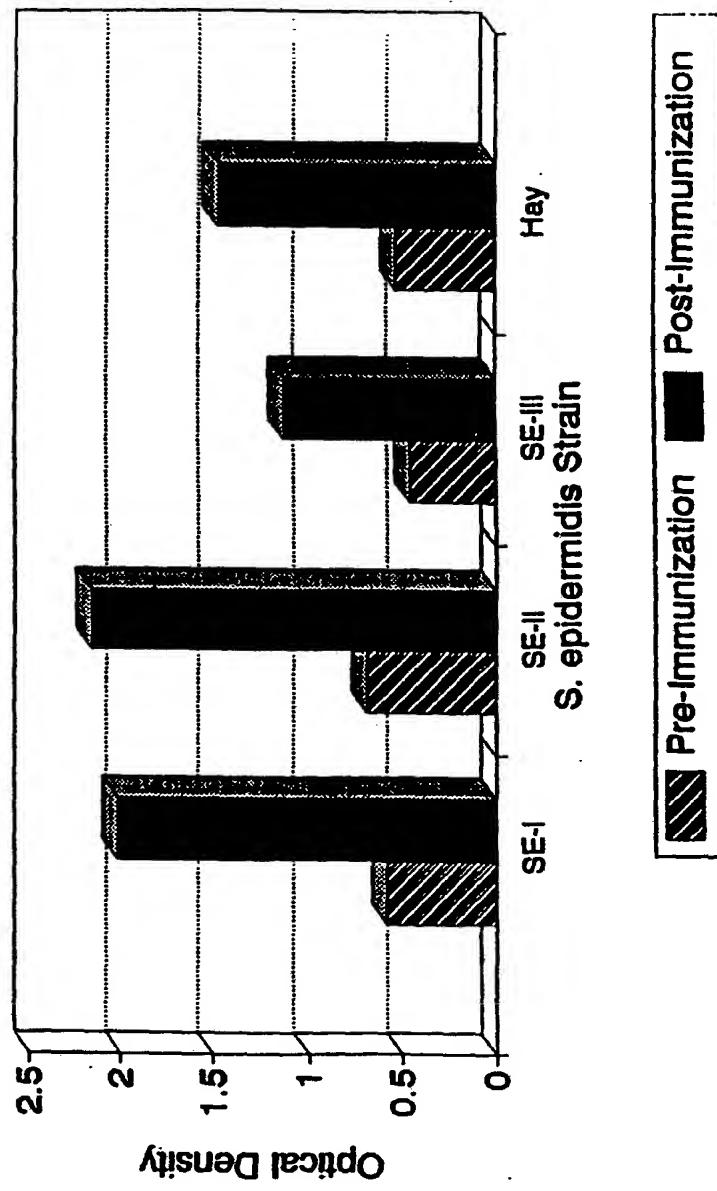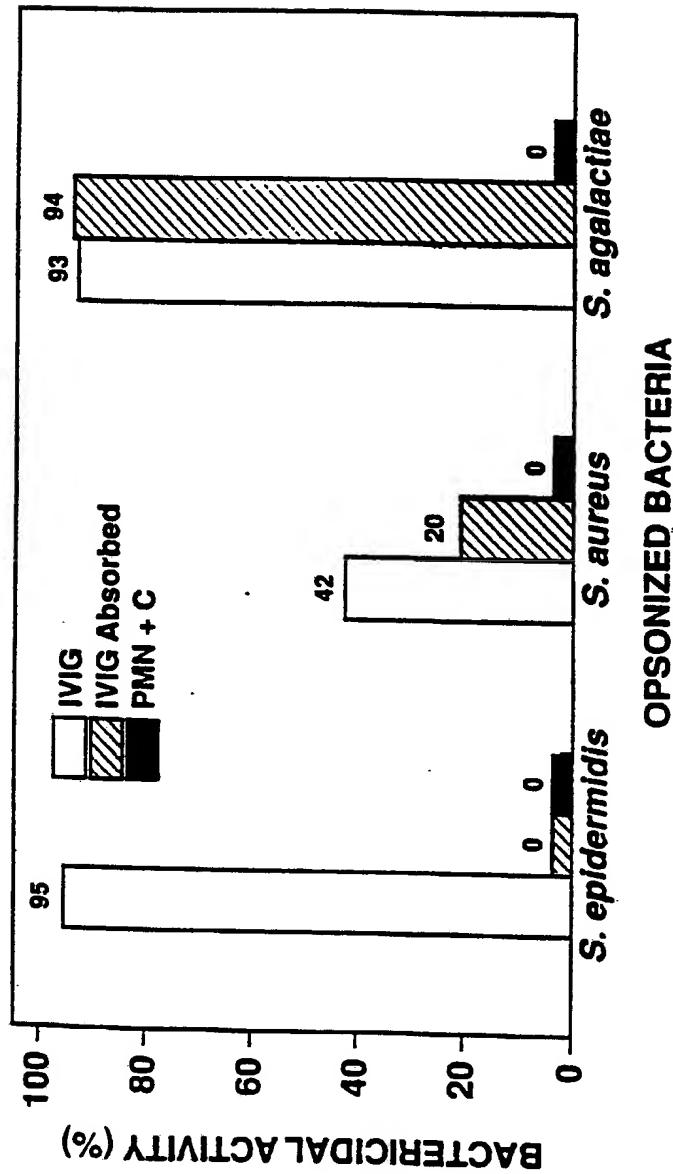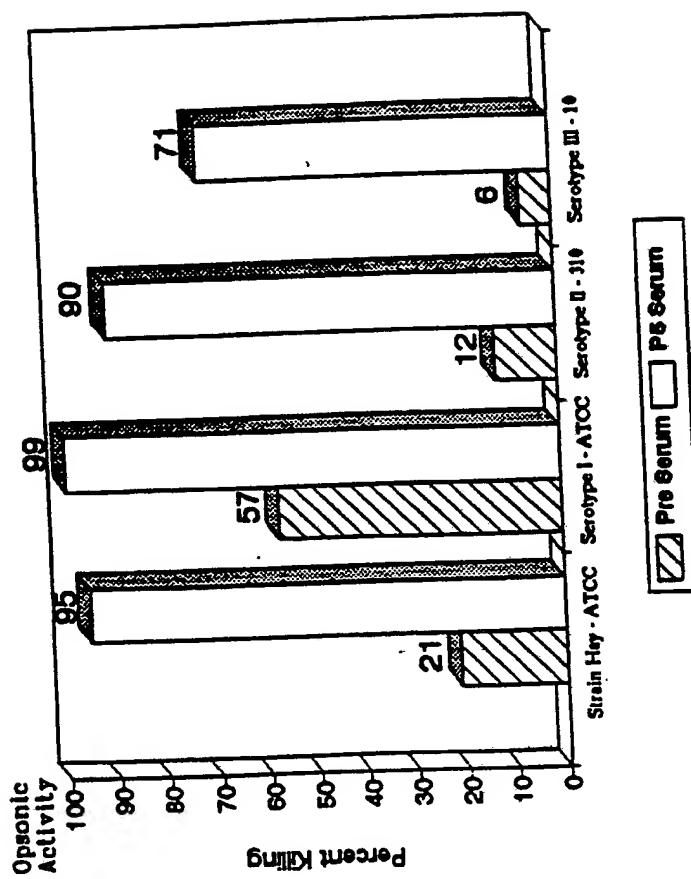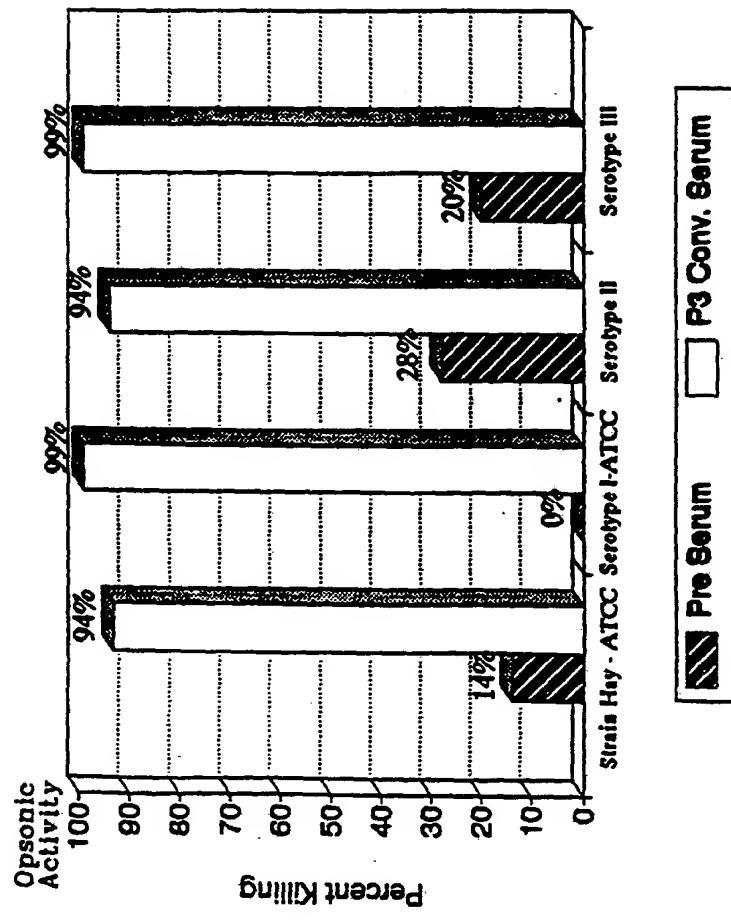


Figure 5

Figure 6

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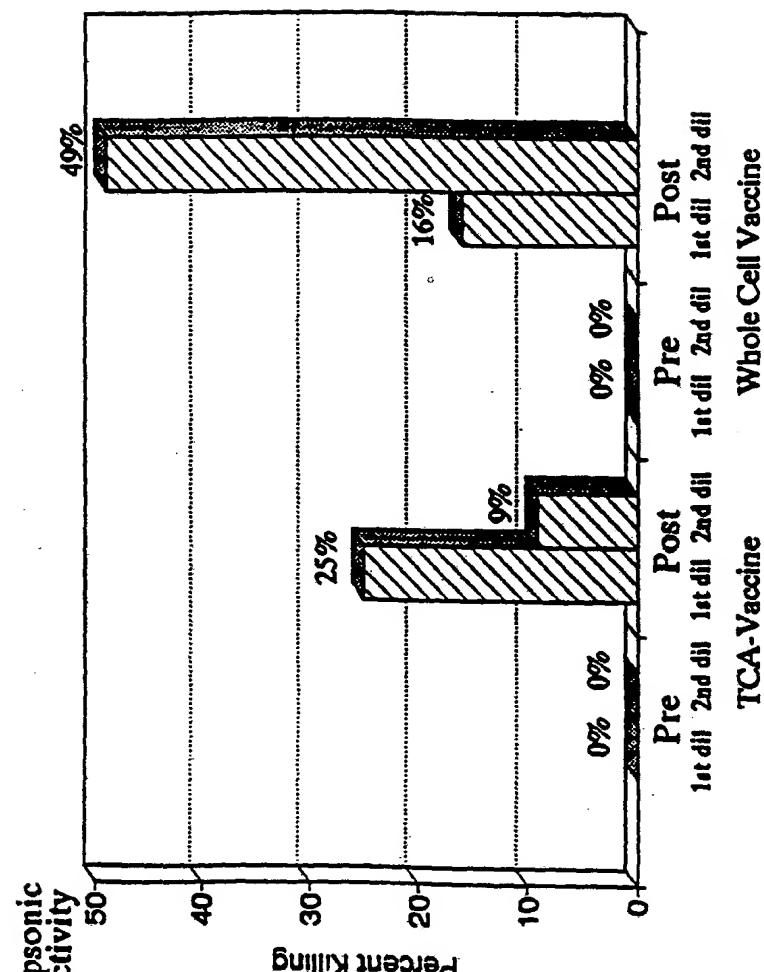
Figure 7

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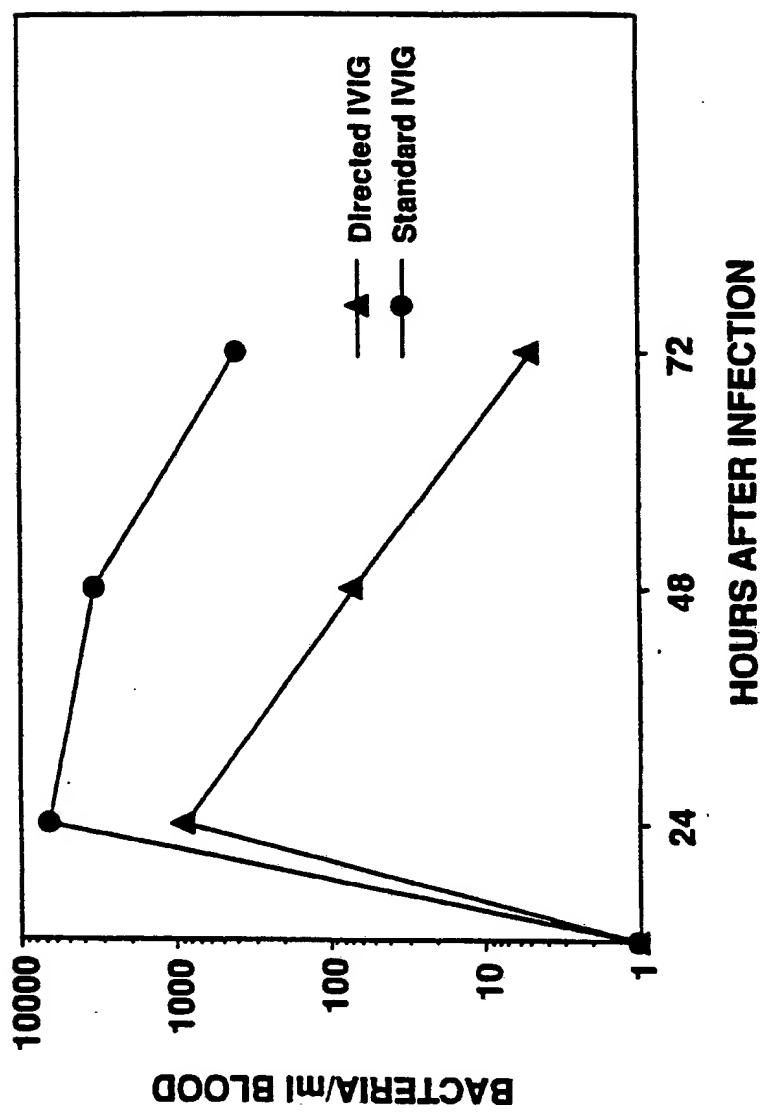
Figure 8

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Figure 9

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Figure 10

p=0.00017, 2-tailed Fisher exact (D-IVIG vs. A-IVIG)

Directed IVIG
Absorbed IVIG
Saline

PERCENT SURVIVAL

DAYS AFTER INFECTION
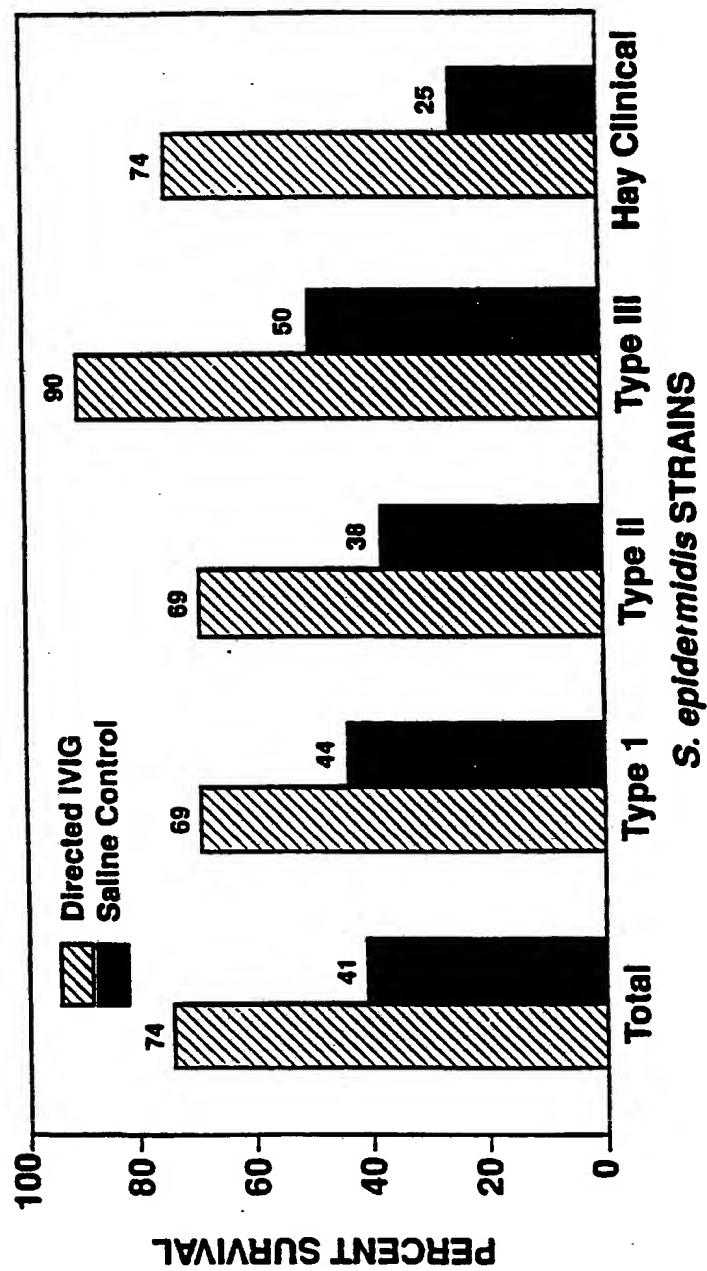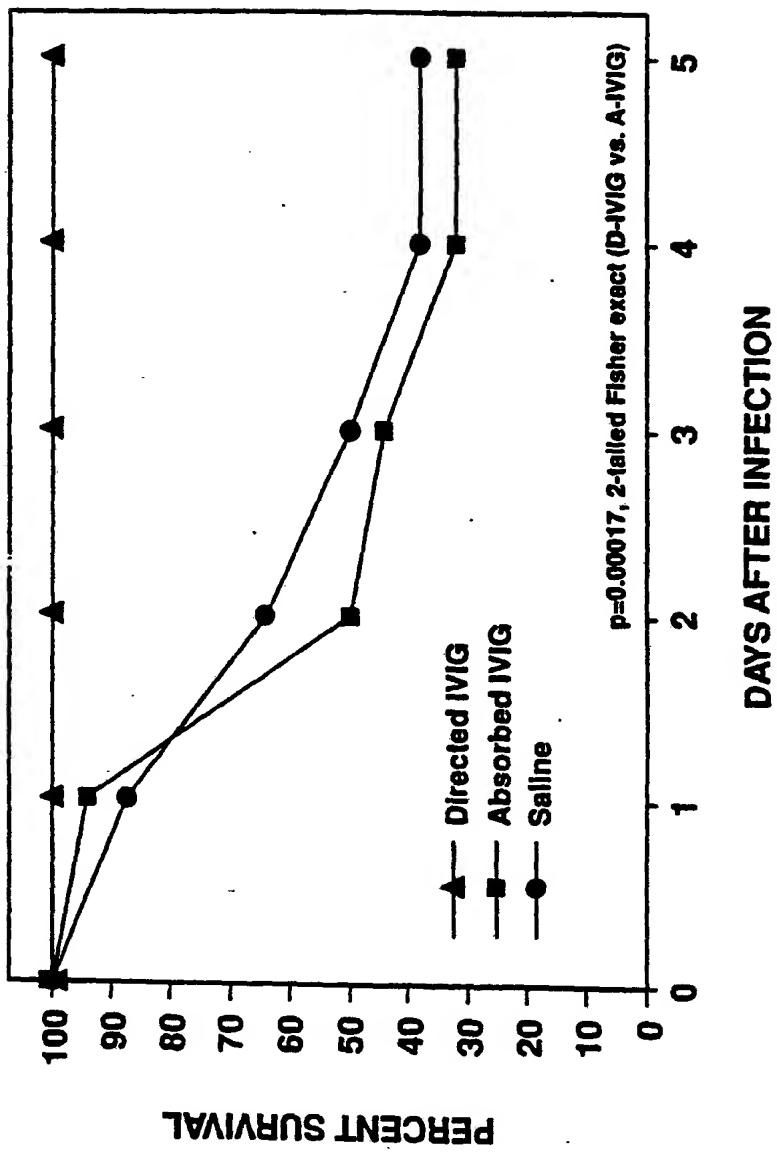
11/12

**Figure 11**

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Figure 12

**I. CLASSIFICATION F SUBJECT MATTER** (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 G01N33/569;      G01N33/577;      C07K15/00;      C12P21/08

**II. FIELDS SEARCHED**

Minimum Documentation Searched[7]

| Classification System | Classification Symbols |
|---|---|
| Int.Cl. 5 | G01N ;     C07K |

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched[8]

**III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]**

| Category[D] | Citation of Document,[11] with indication, where appropriate, of the relevant passages[12] | Relevant to Claim No.[13] |
|---|---|---|
| X | JOURNAL OF MEDICAL MICROBIOLOGY vol. 35, OXFORD UK pages 65 - 71 65-71 'Characterization and functional aspects of monoclonal antibodies specific for surface proteins of coagulase-negative staphylococci.' see page 67; table 1 | 1,2 |
| Y | see whole article | 3-66 |
| Y | PEDIATRIC RESEARCH vol. 29, no. 4, 1 April 1991, NEW YORK NY USA page 281A G.W. FISCHER ET AL. 'Directed immune globulin enhances survival in an intralipid induced neonatal model of lethal Staphylococcus epidermis sepsis.' see whole abstract | 3-66 |

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**IV. CERTIFICATION**

| Date of the Actual Completion of the International Search | Date of Mailing of this International Search Report |
|---|---|
| 08 JULY 1993 | 3 0. 07. 93 |
| International Searching Authority | Signature of Authorized Officer |
| EUR PEAN PATENT FFICE | VAN BOHEMEN C.G. |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT | (CONTINUED FROM THE SECOND SHEET) | |
|---|---|---|
| Category° | Citation of Document, with indication, where appropriate, of the relevant passages | Relevant to Claim No. |
| T | PEDIATRIC RESEARCH<br>vol. 31, no. 4, 1 April 1992, NEW YORK NY USA<br>page 275A<br>T.J. CIESLAK ET AL. 'Post-immunization antibodies to S. epidermidis are broadly reactive and opsonic.' ‑‑‑‑‑ | 1-66 |